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THE CONTROL OF SCHISTOSOMIASIS JAPONICA. III. STUDIES ON THE LONGEVITY OF CERCARIAE OF *SCHISTOSOMA JAPONICUM* IN SALINE SOLUTIONS†

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INTRODUCTION

Among the military command on Leyte, Philippine Islands, in the summer of 1945, there arose the question as to whether ocean bathing near the mouths of the several infested fresh-water streams constitutes a health hazard through possible exposure to cercariae of *Schistosoma japonicum*. Studies by Faust and Meleney (1924) and Magath and Mathieson (1946) have indicated that the intermediate hosts of *S. japonicum* do not survive in habitats proximal to the sea. Working with the Japanese intermediate host, Faust and Meleney demonstrated in the laboratory that a dilution of sea water with an equal part of tap water is lethal to the snail in twenty-four hours. Magath and Mathieson did not find the intermediate host, *Oncomelania quadrasi,* in those swamps of the Coastal Plain of Leyte which are occasionally inundated with water from the sea. Although the fresh-water swamps about the Palo River in East Central Leyte which are infested with the parasite are some way upstream, it is known that cercariae of this parasite are carried for considerable distances from the snail colonies where they are shed; and it is considered possible that some might be transported downstream into the ocean. At the request of the military authorities tests were conducted on two separate occasions to determine the possibility of exposure in the estuarine waters about the mouth of the Palo River, in the San-Pedro San-Pablo Bay area. In June Dr. Ernest Carroll Faust, in cooperation with Capt. Malcolm S. Ferguson, Sn.C., tested the period of survival of cercariae of *S. japonicum* in samples of water taken from the mouth of the River and the apron of the River where it spreads out into the Bay. Further tests were made with offshore waters of the Bay several hundred yards away from the mouth of the River. Cercariae from crushed snails were introduced into eleven test waters and observations were made over a thirty-minute period. Controls were run with pond water. The readings taken indicate that about 1.75% salt concentration in sea water constitutes a critical salinity level and that 3% to 4% is almost instantaneously lethal to the cercariae. The latter degree of salinity was present in

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water offshore about 225 yards above the mouth of the River and extended for several hundred yards beyond. The Bay water was therefore certified as safe for swimming within the limits indicated, and with the stipulation that additional tests for salinity of the water would have to be made whenever heavy rains brought a great volume of fresh water down the River and into the Bay.[1]

The area was re-examined in July by Dr. Faust and Major D. Kirkham, M.C., because there was then high water in the River. Samples were taken along a 1000-yard front on the beach and tested for the degree of salinity. A maximum salt content of 2.27% was found at the point farthest from the river mouth. It was thereupon recommended that no swimming or boating be allowed within one mile of the Palo River.[2]

In September a laboratory study was undertaken by the writer to determine the rate at which various concentrations of sodium chloride (Merck) would accomplish total kill of naturally shed cercariae of *S. japonicum*. Comparisons were made with equivalent concentrations of sea water. The author was assisted in this work by Sergeants Preston M. Bauman and James K. See.

MATERIALS AND METHODS

Sodium chloride solutions were made up in filtered water from the Tanauan River, Leyte, at each half per cent from 1% to 4%. This river was the source of water used in the laboratory by the Commission on Schistosomiasis for hatching eggs of *S. japonicum,* maintaining *Oncomelania quadrasi* snails, and obtaining discharge of *S. japonicum* cercariae from these snails. The sea water used was taken off shore at White Beach, near Tacloban, where it was obtained during a rainstorm and following a day and a half of rain. Such water was believed to be the optimum natural sea water for survival of a fresh-water organism. Dilutions of the sea water were made volumetrically with Tanauan River water and filtered. The specific gravities of the sodium chloride solutions were checked with a Baumé scale and the salt content of each sea-water dilution was calibrated against the known NaCl solutions by use of a salometer. Salinities of the dilutions were approximately as follows: 100% sea water, 3%; 67% sea water, 2%; 50% sea water, 1.5%; and 33% sea water, 1%. The Tanauan River water had a specific gravity of 1.000. Each of the solutions and dilutions had a pH 7.6, as did the Tanauan water and the sea water. These pH determinations were made with a Hellige type color comparator set.

All cercariae used were naturally shed at night in water having a pH of 7.6. The age of these free-living organisms varied from one hour or less to 48 hours. Their viability was much reduced during the 24–36-hour period, and cercariae 36–48 hours old were relatively feeble. On the other hand, preliminary tests indicated that the age of cercariae up to 24 hours does not seem to be important in determining their longevity in saline solutions. In 50% sea water cercariae which had been shed only an hour or less before and those 22 to 24 hours old were equally susceptible and succumbed in five to ten minutes.

For each of the tests in the table below, fifty cercariae less than 24 hours old

[1] Faust, E. C. Progress report on possibility of exposure to schistosomiasis on beach at Base K New Headquarters, San Pedro Bay and Palo River. Unpublished report, 10 June 1945 to the Surgeon, Base K.

[2] Faust, E. C., and Kirkham, D. Schistosomiasis hazard on swimming beach, Hq Base K. Unpublished rept., 28 July 1945, to the Surgeon, Base K.

were counted out into a small quantity of water in a sputum bottle. All of this water was drawn up into a syringe through a hypodermic needle, and then injected into another sputum bottle almost full of the solutions being tested. Controls demonstrated that there was some loss of cercariae in this method, but that the longevity of the cercariae which were successfully transferred was not reduced. Since the primary object was to determine the time for the total kill, i.e., the time interval from the beginning of the test until the last cercariae present had died, the number of cercariae present at initiation of the time interval was not of primary significance. Although some dilution of the test solutions occurred with the introduction of the cercariae, an error in this direction serves as a safety factor in drawing conclusions from the results.

Viable, mature *S. japonicum* cercariae are usually found floating at or swimming near the surface of fresh water. Before they died in the salt water they were observed to descend slowly to the bottom. The criteria for determining their death after the cercariae had reached the bottom of the container were the following: (1) rigidity, (2) reduction in refractile index, and (3) crenation. Darkened and misshapen organisms were watched at least thirty seconds for any sign of mobility, before they were recorded as dead.

The time of introduction of the cercariae was recorded to the nearest five seconds, and counts were made of the numbers alive at 1, 3, 5, 10, 15, 20, 30, 45, 60, 90, and 120 minute periods. The records also included the initial number of cercariae transferred and their age. Observations were made with the high power objective of a dissecting binocular microscope fitted with 15 × oculars. Extreme variation of temperatures was from 26° C to 30° C.

RESULTS

TABLE 1.—*Effect of NaCl on cercariae of* Schistosoma japonicum

Number of tests run	Strength of NaCl solution	Time in minutes required for total kill of *S. japonicum* cercariae			
		Minimum	Mean	Maximum	Minimum rate of kill in ten minutes
10	4.0%	1	1	1	100%
1	3.5%	1	1	1	100%
14	3.0%	1	2	3	100%
10	2.5%	1	3	3	100%
10	2.0%	1	3	5	100%
13	1.5%	1	5	10	100%
6	1.0%	10	60	120	0%

TABLE 2.—*Effect of sea water on cercariae of* Schistosoma japonicum

Number of tests run	Proportion sea water	Salinity	Time in minutes required for total kill of *S. japonicum* cercariae			
			Minimum	Mean	Maximum	Minimum rate of kill in ten minutes
10	100%	3.0+%	1	3	3	100%
14	67%	2.0+%	3	7½	15	80%
9	50%	1.5+%	5	20	90	60%
6	33%	1.0+%	45	105	Over 120	0%

DISCUSSION

Table 1 shows that sodium chloride solutions with a 1.5% or greater salinity were completely effective in killing all cercariae within ten minutes. As the concentration dropped from 1.5% to 1% NaCl, there was a sharp increase in the period

required to assure total kill. In one test with 1% NaCl all cercariae were killed within ten minutes. In another it was 120 minutes before the last cercaria was found to be dead. Only this single cercaria had been alive at the 90-minute interval and it was then at the bottom of the container occasionally twitching feebly. It is extremely doubtful if the cercaria was infective then or had been for some time.

The figures given for the rates of kill with the sea-water dilutions are conservative, and the percentage of cercariae killed by these dilutions during the first ten minutes the organisms were exposed was usually greater than is indicated in Table 2. For example, in thirteen tests out of fourteen, 67% sea water showed an 100% kill within ten minutes. In the fourteenth trial, 20% of the cercariae still survived at ten minutes; but all were dead at fifteen minutes.

A comparison of Tables 1 and 2 shows that both sea water and sodium chloride solutions are almost equally lethal when the salinity is 3%. When the salinities are less than 3%, sea water is considerably less toxic than the corresponding pure sodium chloride solution.

The data show the results of transferring cercariae from fresh water directly into salt water. No work was done on the effect of a gradually increasing salinity, the natural condition encountered by cercariae as they are carried out from a river mouth into the ocean. Likewise, no attempt was made to demonstrate the degree of infectivity of the cercariae living in saline solutions.

Krakower (1940) obtained essentially similar results while working with the cercariae of *Schistosoma mansoni*. He reported that solutions of sodium chloride under 0.6% were without any effect, and that solutions from 0.6% to 1.0% caused the cercariae to drop permanently to the bottom where they remained fairly active or were more restricted in their movements. He found that sodium chloride solutions of 1.5% or stronger rapidly killed the cercariae. Moreover, Faust, Jones, and Hoffman (1934) suggested that cercariae are comparatively ineffectual in accomplishing penetration of the mammalian host unless they are near the surface of the water, and this has been demonstrated by Krakower (1940).

SUMMARY

Insofar as the hazard of exposure to *S. japonicum* cercariae is concerned, sea water with a 3% salinity may be considered absolutely safe for bathing, since it provides complete kill of the cercariae in less than three minutes. Areas in which sea water is diluted to less than 1.5% salinity by river waters infested with these cercariae must be considered as potentially dangerous. The exact point between sea water salinities of 1.5% and 3% where absolute safety is afforded was not determined in these tests. It will depend upon the infectivity of those cercariae able to survive for a period in such solutions.

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OBSERVATIONS ON THE PATHOLOGY OF SCHISTOSOMIASIS JAPONICA: DIAGNOSIS BY RECTAL CRYPT ASPIRATION

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The specific diagnosis of schistosomiasis japonica can be made only by the demonstration of the eggs, classically by stool examination. Numerous papers have described and evaluated the technics commonly used for fecal diagnosis, and these are the accepted procedures for studying suspected cases. Although probably useful under certain circumstances, as in unisexual infections, skin and complement fixation tests are probably without specific diagnostic value.

Ottolina and Atencio (1943) were not satisfied with current methods for diagnosis of schistosomiasis mansoni and found by a review of the literature that previous authors using various stool examination methods had failed to detect 11 to 18 out of every 20 cases of the disease in Venezuela. They found that KOH digests of single biopsies of the rectal mucosa were more effective than serial stool examinations in demonstrating *S. mansoni* eggs, and were able by this technic to demonstrate 11 active infections among 100 persons who were consistently stool negative. Morales and Maldonado (1945) have further developed this technic.

In schistosomiasis mansoni it is known that the eggs are available in the rectal mucosa. The rectum is one of the organs whose tissues contain eggs most frequently and in largest numbers. Lambert and Burke (1928), in discussing autopsy findings in 35 cases of the disease, emphasized the importance of studying blocks from the rectum for the histologic diagnosis of light infections. By the microscopic examination of tissues from 246 histologically proven cases, Koppisch (1941) found that 67% of examined rectums contained eggs, as compared with 74% of livers, 64% of colons (proximal to rectum), 25% of small intestines, and smaller percentages of other organs. In minimal infections eggs were found only in the rectums, colons and livers. In studies of human, rabbit and monkey tissues infected with *S. mansoni,* Kohlschütter and Koppisch (1941) found that there were always more eggs in the rectum than in any other organ, and that they occur in large groups only in the rectum and colon.

It is important to note that *S. mansoni* eggs in the rectal tissues are concentrated in the mucosa. Koppisch (1941) found that most of the eggs lie in the tunica propria, the mucosal capillaries and the mucosal crypts, and that they are found in rapidly diminishing numbers from the mucosa towards the periphery. Kohlschütter and Koppisch (1941) confirmed these findings in human and animal material.

The eggs are able to leave the mucosa rather easily and thus become available for demonstration. Although in most tissues the presence of eggs elicits considerable enveloping reaction, Kohlschütter and Koppisch pointed out that the colonic and rectal mucosa reacts differently than the submucosa, the hepatic and other tissues. In the other coats of the gut there is always proliferation of fibroblasts and accumulation of histiocytes and leukocytes, while in the mucosa the eggs stimulate little or

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no tissue reaction. Koppisch, from his large experience, made this same observation and concluded that fibrosis of the gut mucosa never reaches the point where it could impede the passage of eggs into the lumen.

It has long been an accepted concept that the adult *S. japonicum* inhabits and oviposits in the venous radicles of the superior mesenteric vein, while *S. mansoni* chooses the radicles of the inferior mesenteric vein (Craig and Faust, 1943). The eggs of the former would, therefore, eventually be shed into the lumen of the small bowel and proximal colon, and those of the latter into the distal colon and rectum. It would seem, therefore, that the biologic argument, presented in the previous paragraphs, for the potential availability of *S. mansoni* eggs in the rectal mucosa, would not apply in the case of *S. japonicum*. There is much evidence, however, to indicate that there is no definite anatomic pattern to the mesenteric distribution of the two species. Thus, Koppisch (1941) stated: "In heavy infections (with *S. mansoni*) the worms tend to occupy veins draining the higher levels of the intestine, including the small gut, and even branches of the portal that drain such organs as the spleen, pancreas and stomach." That *S. japonicum* eggs are deposited in and emerge from the gastro-intestinal tract as low as the rectum, even in very early and presumably light infections, has been adequately demonstrated (Johnson and Berry, 1945).

It is generally recognized that scraping or aspirating typical rectal ulcers affords material in which the identification of trophozoites of *Endamoeba histolytica* is facilitated. An aspiration technic has been used for several years by the senior author and, in accord with the experience of others, has reduced the time required to establish the specific etiology of such rectal lesions. The present study was initiated because the diagnosis of schistosomiasis was presenting an administrative problem due to the tedious and time-consuming procedure of multiple stool examinations. The sigmoidoscopic picture in certain schistosomiasis suspects suggested the application of the aspiration technic to facilitate the specific diagnosis of the disease.

MATERIAL

Soldiers coming under our care had been evacuated for reasons other than schistosomiasis and had reached the terminus of the chain of evacuation, a general hospital in the Zone of the Interior. Those who were schistosomiasis suspects were studied by 36 serial stool examinations and six sigmoidoscopies with rectal crypt aspiration. Twenty-four patients were submitted to this routine; 13 had served on Leyte and were studied from 14 to 16 months after their first exposure to known infectious water, and 11, who had worked in the rice fields of Mindanao as Japanese prisoners, were studied 44 months after first exposure.

METHODS

Rectal crypt aspiration (modified technic of Felsen, 1938).—Sigmoidoscopic examination was performed following a bowel movement, without purgation or enema. By means of a heavy-walled bent-tipped glass pipette connected with a motor-driven suction pump developing 20 inches of mercury vacuum, any suspicious lesion was thoroughly scraped and aspirated. In the absence of lesions, the suction was applied to large areas of the normal rectal mucosa. Collected material was washed back into a vial with tap water. The suspension was later spun down, and the sediment pipetted to a slide; all material under one 22 by 22 mm cover slip was examined.

Stool examination.—Whole stools were passed without purgation into glass jars and examined within four hours. Each specimen was submitted to three technics. Scrutinization of all material under one 22 by 22 mm cover slip constituted the examination for each technic.

1. A zinc sulfate concentration technic (Otto, Hewitt and Strahan, 1941) was used for the detection of parasites other than schistosomes. A suspension of feces in zinc sulfate solution of specific gravity 1.180 was allowed to stand for 30 minutes and material from the surface film examined.

2. Direct smear was examined of material selected from the surface of formed specimens and at random from mushy or liquid ones. Flecks of mucus or blood were selected when available.

3. Sedimentation by centrifugalization: a tap water fecal suspension was spun down about four times (until the supernatant was relatively clear), and a portion of the sediment pipetted to a slide and examined.

RESULTS

Seven of the 24 suspects were proved to be infected by either or both diagnostic methods. Four of the seven were diagnosed by rectal crypt aspiration alone, after an average of 4.5 stools from each had been negative (Table 1). Three cases were

TABLE 1.—*Comparison of results of stool examination and rectal crypt aspiration in patients proven infected by either method, prior to the institution of treatment*

Case	Stool examinations		Aspirations	
	Number	Number positive	Number	Number positive
1	4	0	1	1
2	6	3	1	1
3	7	0	2	2
4	5	0	1	1
5	4	1	2	2
6	3	1	2	1
7	2	0	2	2
Totals	31	5	11	10
Per cent positive	..	16	..	91

found positive both by stool examination (4.3 per patient) and rectal crypt aspiration (1.7 per patient). In the series, five of 31 stools and 10 of 11 aspirations were positive. Seven of the 10 positive aspirations were taken from normal appearing rectal mucosa. In each of the seven patients the first aspiration was positive.

The sigmoidoscopic picture.—The seven proved active cases were submitted to a total of 31 sigmoidoscopic examinations. In three patients the examinations (three apiece) were consistently normal. In the other four the serial examinations before and during treatment were marked by a variety of quickly changing superficial mucosal lesions, from the distal rectum to the limit of the visualization at 25 cm. From time to time the examinations were normal. It is notable that the institution of tartar emetic treatment effected no trend toward improvement in the observed lesions until more than about 0.4 gm of antimony had been given. The most commonly observed lesions were ephemeral clusters of two to four bright 1 by 1 mm dry petechiae in an otherwise normal mucosa; the petechiae were located 5 to 25 cm above the sphincter, showed no constant relationship to the vascular pattern and did not blanch on pressure. Other pathology observed consisted of dull patches with a sandpaper-like appearance, superficial erosions, stellate superficial ulcers, hyperemic easy-bleeding areas, and, in a single instance, a 1 by 1 cm firm yellow plaque.

Fifty-five sigmoidoscopic examinations were made on 17 schistosomiasis suspects who were not found to have infections. Ten of these patients, who were submitted to an average of three examinations, had consistently normal pictures. The other seven showed minor pathology including lymphoid hyperplasia, benign rectal polyps or groups of petechiae indistinguishable from those seen in proved schistosomiasis cases.

DISCUSSION

The disadvantages of fecal examination for the diagnosis of the enteral schistosomiases are the laboratory time consumed in the examination, the large number of stools which must often be examined from a single patient before a positive is found, the delay in institution of therapy thus entailed, and, most important, the possibility of missing an active infection by discontinuing study too soon. These difficulties have been adequately illustrated by Hesselbrock et al (in press). Although it was felt that rectal biopsy was a safe and, from reports, an effective procedure, simple aspiration seemed the preferable technic because eggs from a far greater area of the mucosa could thus be collected. The sigmoidoscopy and aspiration require only about five minutes. The apparatus involved is simple and easily improvised. It is probable that suction applied by a heavy-walled rubber bulb would be as effective as that of a vacuum pump, although less facile.

CONCLUSION

The small series of cases described here indicate that rectal crypt aspiration is a more reliable and time-saving technic for diagnosing schistosomiasis japonica than the stool examination methods used. We feel that the described technic may eventually prove to be the method of choice for diagnosing the enteral schistosomiases.

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EFFECTS OF DDT ON CERCARIAE OF *SCHISTOSOMA MANSONI**

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The toxicity of DDT (dichloro-diphenyl-trichloroethane) to the arthropods in general is quite well known, but information concerning its toxicity to other invertebrates is comparatively scarce. Because of the extensive use of DDT in malaria control, and since malaria and schistosomiasis are frequently encountered in the same areas, it has seemed desirable to determine to what extent DDT could be relied upon for emergency schistosomiasis control; i.e., for killing cercariae and thus providing temporary protection for military personnel in endemic schistosome regions.

MATERIALS AND METHODS

Tests were concerned primarily with the cercaricidal properties of xylene-Triton emulsion concentrate (used as a vehicle for DDT dispersion) with and without DDT, and Triton (Triton X–100) at dosages which are practical in the field. Additional tests were performed to demonstrate the toxicity of: (a) DDT-xylene-Triton and xylene-Triton emulsion concentrates which were diluted and aged for a period prior to use; (b) crystalline or powdered DDT in water; and (c) DDT applied in oil films on the surface of water. In the course of these studies questions arose concerning the viability and infectivity of schistosome cercariae affected by treatment. Consequently, supplemental experiments were designed to clarify the contention that: (d) a certain percentage of cercariae affected by treatment with DDT-xylene-Triton emulsion concentrates are capable of infecting a host; and (e) cercariae treated with DDT-xylene-Triton and xylene-Triton emulsion concentrates, and then revived by the introduction of fresh water, are also infectious.

Cercariae.—Cercariae of the trematode, *Schistosoma mansoni* Sambon 1907 were obtained from the snail intermediate host, *Australorbis glabratus* Say 1818 procured from Puerto Rico, and raised and infected in the laboratory. Washed snails were placed in spring water containing sufficient calcium carbonate to maintain a hydrogen ion concentration of approximately pH 7.0, previous observers having found that cercariae are most frequently found in water with such characteristics (Hoffman and Faust, 1934). Water (0.2–0.4 ml) containing the schistosome cercariae was transferred to 30-ml capacity "petroleum test" dishes for testing purposes.

Definitive laboratory host.—The golden hamster (*Cricetus auratus*) has proved to be a satisfactory laboratory host for *S. mansoni.* Hamsters were used in all experiments in which the infectivity of emulsion-treated schistosome cercariae was studied.

Dosages of emulsion concentrates and component parts tested.—In order to have standard dosages for test materials which would be comparable in the field and laboratory work, Table 1 (below) has been formulated giving the dilutions used in the laboratory and their approximate equivalents in rates applied in the field. Cal-

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culations for the values given were based upon the ratio of weight of DDT, xylene-Triton emulsion or Triton to the weight of an acre of water one foot in depth. Gallons per acre foot of water has been used as the unit of measurement since treatment of water with emulsions is concerned with more than surface and related surface phenomena. The emulsion concentrate tested was that prescribed for general field application; i.e., DDT (technical trade) 20 gm; xylene, 60 ml; Triton X–100, 20 ml.

Dilution of emulsion concentrates and Triton.—Emulsion concentrates of DDT-xylene-Triton (DDT, 20 gm; xylene, 60 ml; Triton, 20 ml) and xylene-Triton (xylene, 60 ml; Triton, 20 ml) were diluted just prior to use in each experiment except in special tests which were performed to determine the cercaricidal properties of the stock water emulsion, which had been aged for some time before final dilution. At the beginning of each experiment a 10-ml volume of the preparation to be tested was transferred to petroleum test dishes containing 0.2–0.4 ml of cercarial suspension.

Crystalline DDT.—For the determination of cercaricidal effects of crystalline DDT, a volume of solvent was used which contained dissolved DDT equivalent to the weight present in an equal volume (10 ml) of test emulsion at the rate of 20 gal./acre ft. Two series of experiments were run, one using xylene as the solvent

TABLE 1.—*Equivalent concentrations of components tested*

Concentration of DDT-xylene-Triton emulsion in water	Concentration of xylene-Triton emulsion in water	Concentration of Triton in water	Equivalent rates of application used in field work
1 : 16,300	1 : 19,200	1 : 72,100	20 gal./acre ft.
1 : 32,600	1 : 38,400	1 : 144,200	10 gal./acre ft.
1 : 65,200	1 : 76,800	1 : 288,400	5 gal./acre ft.

and the other, acetone. The required amount of DDT dissolved in 1 ml of the solvent was deposited on the sides and bottom of the test dishes and the solvent was allowed to evaporate and dry. Test dishes were kept on a wire frame above an electric hot plate from several hours to several days before they were used to insure highest possible evaporation of solvent and crystallization of the DDT. Regardless of technic and care employed, droplets of a supersaturated solution of DDT in xylene and in acetone often remained on the bottom and sides of the test containers. This was not surprising, as previous work (Richards, Cutkomp and Morton, 1944) has shown that such droplets form, leaving 1–10 per cent of the DDT in droplets as a result of the impurities in the DDT.

Oil and DDT-oil films.—Saturated solutions of DDT in xylene, No. 2 diesel oil, mineral oil and lubricating oil (SAE 30) were allowed to stand for several days at room temperature of 23–25° C. For testing purposes 7–15 drops of DDT in the various solvents were dropped on the surface of a water suspension of cercariae in petroleum test dishes, making a thin film covering the entire water surface. In several exploratory tests on very thin films of the oils, an ordinary household hand sprayer was employed to dispense the preparations as very light, just visible films on the water.

Method for counting cercariae.—Since the effects of cercaricides were ascertained by direct observation of loss of motility, there was need for graded categories to indicate the condition characteristic of the cercariae at the time of examination. Schistosome cercariae in contact with toxic agents show a gradual but definite loss of ability to swim freely followed by progressive inhibition of body movements, and

finally, complete absence of movement as the animals approach death. These stages have been designated by letters A, B, and C. (A) denotes cercariae which are apparently normal, very active, and capable of swimming freely throughout the test medium. (B) includes all cercariae whose actions are impeded to the extent that they are unable to move freely, both those which are only slightly affected and those which are very weak and barely capable of muscular contraction and body movement. Cercariae in stage C show no movement and have an opaque appearance, lacking the high refractivity of light characteristic of living cercariae. With death, also, there is a relaxation of the muscles which maintain rigidity of the acetabulum and the tail, accompanied by a loss of tonicity of the body musculature as a whole.

In experiments for testing cercaricidal properties of xylene-Triton emulsions with and without DDT, observations were made at 15-minute intervals on three test containers, one dish of cercariae in diluted emulsion concentrate containing DDT, one dish without DDT and one control dish with cercariae in spring water. Tests to determine the toxicity of Triton were run separately. Examinations were continued until all the cercariae were dead (stage C) in dishes with the toxic compounds. In some tests the control dishes were followed until death claimed all cercariae. In counting, the number of cercariae classed as condition B and C was recorded, then the number in stage A was determined by substraction, after the total in each dish was known. Experience has shown that accurate counts cannot be made on large numbers of free-swimming cercariae.

In all other tests concerned with the killing properties of DDT, observations were made at $\frac{1}{2}$-hour intervals during the first three hours, then at one-hour intervals. Water control dishes with schistosomes in untreated water were examined in each experiment, and a series of tests was carried out to determine the normal longevity of cercariae in spring water under laboratory conditions. These observations were made at two-hour intervals, the data being recorded as in cercaricide tests.

Infection of hosts with treated and untreated cercariae.—To determine the expected return of worms from hosts exposed to untreated cercariae, unclipped hamsters were kept for four to six hours in culture dishes holding 25–50 ml of water and a calculated number of schistosome cercariae.

To ascertain the infectivity of treated cercariae, large numbers of cercariae (estimated by a sample count) were placed for one hour in emulsions of DDT-xylene-Triton and xylene-Triton in test dishes, then one hamster was immersed in each container for 14–16 hours to allow maximum infection by the affected (stage B) cercariae. For conditions even more favorable to infection, anesthetized hamsters were placed belly up and exposed to a suspension of similarly treated cercariae in a chamber formed by holding a 1-inch length of $\frac{3}{4}$-inch glass tubing against the clipped belly of the hamster.

Experiments to determine the infectivity of schistosome cercariae treated with test emulsions and then revived in fresh water were essentially similar to those just described, except that 250-ml conical glasses were used to facilitate handling of the cercariae, and the test emulsions applied at rates of 20, 10 and 5 gal./acre foot were drawn off and replaced by an equal volume of spring water at the end of one hour. Unshaved hamsters were then exposed to these revived cercariae as they were to those which were only treated.

Recovery of parasites from the infected hosts.—For recovery of parasites, the exposed hamsters were etherized, dissected and the young worms removed. Care

was taken to obtain as much blood as possible when removing the lungs and liver. These organs were placed in physiological salt solution in Petri dishes and examined under the dissecting microscope as each piece of tissue was thoroughly macerated. The number of worms present was then determined.

Examinations for worms were made 10–15 days after exposure of the host to the cercariae of *S. mansoni*. A more accurate count was obtained at the end of this time interval than at the end of 35–40 days at which time adult flukes may be recovered. Experience in this laboratory has shown that after 12–14 days there is destruction of a certain percentage of the worms in the lungs and in the liver, or in transit from the former to the latter as was suggested by Krakower, Hoffman and Axtmayer (1940).

RESULTS

Longevity or survival time recorded for normal untreated cercariae.—Cercariae allowed to die a natural death in untreated water exhibit variations of considerable magnitude in duration of life, the natural survival time varying with lots of cercariae from different snails and with conditions under which they are shed. These differences are due, perhaps, to inherent qualities of the cercariae as well as to varying temperatures and bacterial populations present in cultures. Examinations made at hourly intervals revealed that the time required for the death of all untreated cercariae in a test dish ranged from a minimum of 40 hours to a maximum of 72 hours with an average of 50 hours. These findings are in accordance with those of Krakower (1940) who stated, "there is reason to believe that different lots of cercariae react in a markedly different manner toward injurious agents, with regard to the duration of life." The evaluation of compounds tested and the death rate for untreated cercariae, i.e., the water controls, are of necessity based upon data from a number of experiments.

Schistosome cercariae treated with DDT-xylene-Triton and xylene-Triton emulsion concentrates, and Triton.—DDT-xylene-Triton emulsion concentrate at the rate of 20 gal./acre ft. of water affected the majority of the cercariae within 15 minutes after contact and killed all of the organisms in two and one half hours (Table 2 and Fig. 1). Cercariae treated with this emulsion concentrate at 10 and 5 gal./acre ft. were all dead by the end of 6 and 14 hours respectively.

Tests run simultaneously, using cercariae shed from the same snails revealed that the xylene-Triton emulsion concentrate without DDT at the rate of 20 gal./acre ft. killed all the cercariae within eight hours (Table 2 and Fig. 1), while nearly all were stupefied, i.e., classified as stage B, within 15 minutes. At rates of 10 and 5 gallons of concentrate per acre foot of water all the cercariae were not dead until the end of 22 hours and 30¾ hours, respectively.

The emulsifying agent, Triton X–100, applied at the rate of 20 gal./acre ft. of water also immobilized practically all the cercariae by the end of the first 15-minute period, and all the schistosomes were dead eight hours after the application of Triton as was recorded also for the xylene-Triton emulsion concentrate (Table 3). Triton at one-half the above rate did not kill all the cercariae until 48 hours after the beginning of the test.

Xylene-Triton emulsion concentrate with and without DDT at the greatest test dosage stupefied the animals and caused them to drop to the bottom of the test containers almost immediately. Triton, alone exhibited this same characteristic.

TABLE 2.—*Relative toxicity of test dosages of xylene-Triton and DDT-xylene-Triton emulsion concentrates to cercariae of* S. mansoni

Time of observation (hours)	Concentration of xylene-Triton									Concentration of DDT-xylene-Triton								
	20 gal./acre ft.			10 gal./acre ft.			5 gal./acre ft.			20 gal./acre ft.			10 gal./acre ft.			5 gal./acre ft.		
	Condition of cercariae*			Condition of cercariae			Condition of cercariae			Condition of cercariae*			Condition of cercariae			Condition of cercariae		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
¼	0.1	95.5	4.4	2.6	96.8	0.6	98.9	1.1	0.0	0.0	96.0	4.0	2.5	97.5	0.0	99.2	0.8	0.0
½	0.0	83.7	16.3	2.0	95.5	2.5	97.2	2.8	0.0	0.0	84.7	15.3	0.7	98.5	0.8	96.5	3.5	0.0
¾	0.0	72.3	27.7	1.7	89.3	9.0	95.2	4.7	0.1	0.0	76.3	23.7	0.0	98.3	1.7	94.5	4.8	0.7
1	0.0	53.7	46.3	1.6	74.6	23.8	92.2	7.1	0.7	0.0	56.0	44.0	0.0	98.1	1.9	69.7	29.6	0.7
1½	0.0	36.4	63.6	1.1	64.2	34.7	76.3	22.3	1.4	0.0	31.0	69.0	0.0	93.0	7.0	21.7	76.5	1.8
2	0.0	23.0	77.0	0.9	61.9	37.2	71.0	27.2	1.8	0.0	5.5	94.5	0.0	87.3	12.7	14.9	83.0	2.1
2½	0.0	17.9	82.1	0.3	59.4	40.3	68.5	28.6	2.9	0.0	0.0	100.0	0.0	74.5	25.5	12.9	82.0	5.1
3	0.0	15.3	84.7	0.3	57.8	41.9	63.3	33.3	3.4	..	...	....	0.0	40.4	59.6	0.0	92.2	7.8
4	0.0	10.3	89.7	0.0	51.7	48.3	40.7	55.5	3.8	..	...	....	0.0	18.0	82.0	0.0	78.9	21.1
5	0.0	5.4	94.6	0.0	48.0	52.0	39.4	56.3	4.3	..	...	....	0.0	1.0	99.0	0.0	63.9	36.1
6	0.0	1.2	98.8	0.0	45.9	54.1	27.7	67.3	5.0	..	...	....	0.0	0.0	100.0	0.0	22.3	77.7
8	0.0	0.0	100.0	0.0	36.1	63.9	24.4	70.1	5.5	..	...	....	..	...	....	0.0	3.7	96.3
10	..	...	....	0.0	32.3	67.7	18.7	74.9	6.4	..	...	....	..	...	....	0.0	2.2	97.8
12	..	...	....	0.0	23.7	76.3	17.6	74.9	7.7	..	...	....	..	...	....	0.0	0.6	99.4
14	..	...	....	0.0	16.6	83.4	13.6	77.3	9.1	..	...	....	..	...	....	0.0	0.0	100.0
16	..	....	....	0.0	7.0	93.0	8.4	81.3	10.3									
22	..	...	....	0.0	0.0	100.0	2.5	63.5	34.0									
30¾	..	...	....	..	...	....	0.0	0.0	100.0									

* Condition of cercariae is indicated as the percentage of the total population tested and the cercariae are classed as, unaffected (stage A), affected (stage B), and dead (stage C).

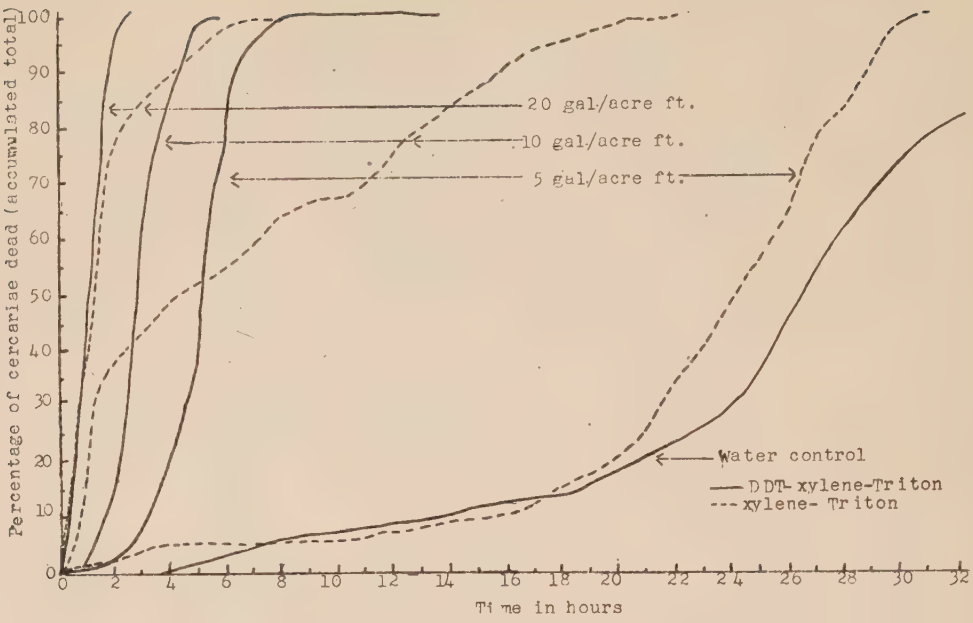

FIG. 1.—Relation of DDT-xylene Triton and xylene-Triton emulsion concentrate dosages to time required to kill cercariae of *S. mansoni* expressed as accumulated percentage of cercariae dead.

Cercariae treated with DDT emulsion concentrate, however, died in a much shorter time. Some of those exposed to xylene-Triton, although immobilized quickly, died at a rate below that recorded for some series of the water controls (Fig. 1).

Data from several experiments in which xylene-Triton was tested at 10 and 5 gal./acre ft. indicated that a small percentage of the total population of cercariae

TABLE 3.—*Relative toxicity of varying dosages of concentrated Triton to Cercariae of* S. mansoni

Time of observation (hours)	Concentration of agent					
	20 gal./acre ft.			10 gal./acre ft.		
	Condition of cercariae*			Condition of cercariae		
	A	B	C	A	B	C
¼	0.0	99.8	0.2	99.7	0.3	0.0
½	0.0	99.6	0.4	99.5	0.5	0.0
¾	0.0	99.5	0.5	99.4	0.6	0.0
1	0.0	99.4	0.6	98.0	1.7	0.3
1½	0.0	95.8	4.2	94.1	5.6	0.3
2	0.0	94.0	6.0	87.0	12.4	0.6
2½	0.0	85.7	14.3	55.8	43.5	0.7
3	0.0	70.4	29.6	27.1	72.1	0.8
4	0.0	32.0	68.0	18.3	80.8	0.9
5	0.0	4.1	95.9	1.4	97.6	1.0
6	0.0	1.2	98.8	0.8	97.6	1.6
8	0.0	0.0	100.0	0.0	97.7	2.3
10	..	...	....	0.0	97.5	2.5
12	..	...	....	0.0	95.5	4.5
14	..	...	....	0.0	94.6	5.4
16	..	...	....	0.0	94.6	5.4
23	..	...	....	0.0	92.7	7.3
25	..	...	....	0.0	88.3	11.7
29	..	...	....	0.0	75.2	24.8
45	..	...	....	0.0	2.2	97.8
48	..	...	....	0.0	0.0	100.0

* Condition of cercariae is indicated as the percentage of the total population tested and the cercariae are classed as, unaffected (stage A), affected (stage B), and dead (stage C).

was first immobilized by the emulsion concentrate, but, adjusting to the medium, recovered sufficiently to become free-swimming again. However, they never actually regained their normal vitality. This phenomenon of conditioning or acclimatization with subsequent increase in vigor of a small part of the cercarial population, after the initial "knock down," may well account for the unusual relationship existing between the survival time of cercariae treated with xylene-Triton emulsion concentrate at 5 gal./acre ft. and the water control (Fig. 1).

Loss of toxicity of DDT-xylene-Triton and xylene-Triton emulsions due to aging.—The first dilutions of DDT-xylene-Triton and xylene-Triton emulsion concentrates, i.e., 1 part of the concentrate to 163 ml and 192 ml of water, respectively, were made and used as stock emulsions for several days. Obvious crystal formation in some of the test dishes of aging DDT emulsion pointed to a direct ratio between the amount of crystallization and the age of the emulsion. As data accumulated, there was evidence, too, of a correlation between cercaricidal properties and age of the emulsion, especially in experiments concerned with cercarial kill by emulsions tested at the rate of 20 gal./acre ft. of water. Thus emulsions of DDT-xylene-Triton applied immediately after preparation required only $1\frac{1}{2}$ hours to kill all cercariae, whereas an emulsion 48 hours old did not kill all cercariae until the end of $6\frac{1}{2}$ hours. Freshly prepared xylene-Triton emulsion killed all cercariae at $2\frac{1}{2}$ hours but the aged emulsion (48 hours) was fatal to the last cercaria only after $9\frac{3}{4}$ hours.

In the xylene-Triton emulsion concentrate the reduction in toxicity may be associated with loss of xylene alone, whereas with DDT-xylene-Triton concentrate it may be due to loss of xylene and subsequent recrystallization of DDT as a result of evaporation. The evaporation of xylene from both emulsions was indicated by the loss of xylene odor and the accumulation of DDT crystals with the increased age of the preparations.

Crystalline DDT.—There was a striking difference in the time required to kill cercariae exposed to DDT recrystallized from acetone and from xylene solutions. In the case of the former, cercariae were killed in five to eight hours with an average kill at $6\frac{1}{2}$ hours in eleven tests, whereas with DDT recrystallized from a xylene solution an average of ten trials revealed that all the cercariae were not dead until approximately 24 hours after their introduction into the test containers.

Even after a very adequate drying period to allow complete evaporation of the solvents, droplets of a supersaturated solution of DDT in acetone or xylene were noticed adhering to the bottom and sides of the test dishes, in addition to the expected crystals of DDT.

Such results are in line with the physical properties of the two solvents and crystallized DDT as explained by Richards, Cutkomp and Morton (1944). Acetone is soluble in water, and therefore a supersaturated solution with DDT yields a definite cercaricidal action when brought into contact with the animals. Xylene is insoluble in water, and consequently droplets of supersaturated solution of DDT remain on the bottom of the test dish and do not disperse when the cercarial suspension is added.

The presence of excessive quantities (2 gm/100 ml water) of powdered DDT sifted into water requires as long as five hours to kill all cercariae since DDT is insoluble in water. Whether death of the cercariae is due to chemical reactions or to traumatic injury by the presence of the many crystals is not known.

Oil and DDT-oil films.—Preliminary tests with oils and DDT-oil solutions indicated the relative ineffectiveness of these agents for cercaricidal purposes. Therefore only Xylene and No. 2 diesel oil, both comparatively toxic, and kerosene, mineral and lubricating (SAE 30) oils, comparatively less toxic, were used with and without DDT.

A film of xylene covering the water surface in test dishes killed the majority of the schistosome cercariae in $\frac{3}{4}$ to $1\frac{1}{2}$ hours and its cercaricidal effectiveness was not noticeably increased by the addition of DDT. A similar film of No. 2 diesel oil killed all of the animals in six to seven hours, and the addition of DDT to the oil reduced the time for complete kill to about $4\frac{1}{2}$ hours. Kerosene on the water killed the cercariae after nine to ten hours exposure; with the addition of DDT complete kill was effected in seven hours. A cercarial suspension treated with mineral oil was nearly normal after 24 hours; a DDT-mineral oil solution was lethal in eight hours. Lubricating oil, with and without DDT, had little effect upon cercariae up to 20–24 hours.

Infectiveness of affected and revived schistosome cercariae.—As investigations on this problem progressed, treated cercariae were suspected of infective potentialities. Moreover, cercariae exposed to and immobilized by xylene-Triton and DDT-xylene-Triton emulsion, and then revived by contact with fresh water recovered sufficiently to swim. This suggested the possibility of penetration should a host be present. The exposure experiments demonstrated the ability of affected and revived cercariae to infect a definitive host.

(a) *Recovery of worms from hosts infected with untreated cercariae.*—Upon examination of nine control hamsters exposed to untreated schistosome cercariae the expected return of worms from these hosts was found to range in individual animals from 6.07 to 19.46 per cent with a total average of 13.30 per cent (Table 4). The infectiveness of cercariae and the subsequent survival of worms in the laboratory host varied as much as the longevity of different batches of untreated cercariae. Although this range is seemingly wide, it is in accordance with the investigations of Krakower, Hoffman and Axtmayer (1940) who have used white rats as the definitive hosts for *S. mansoni.*

TABLE 4.—*Worms recovered from hamsters exposed to untreated, treated, and treated and revived cercariae of* S. mansoni

Hamsters exposed to cercariae		No. of tests	Ave. No. of cercariae per host	Ave. No. of worms recovered per host	Range of recovered worms per host	Ave. percentage of recovery
Untreated (controls)		9	10,536.6	1401.4	369–2,900	13.30
Treated with						
DDT-xylene-Triton	20 gal./acre ft.	3	10,500	13.0	0–27	0.12
	10 gal./acre ft.	3	6,300	15.6	0–28	0.24
	5 gal./acre ft.	2	7,500	100.0	27–173	1.33
Xylene-Triton	20 gal./acre ft.	3	9,500	12.3	0–21	0.13
	10 gal./acre ft.	3	9,300	28.3	0–56	0.30
	5 gal./acre ft.	2	4,000	197.0	136–258	4.92
Revived after treatment with						
DDT-xylene-Triton	20 gal./acre ft.	10	8,210	7.7	0–19	0.09
	10 gal./acre ft.	12	11,800	185.2	9–615	1.57
	5 gal./acre ft.	9	4,400	135.9	79–265	3.09
Xylene-Triton	20 gal./acre ft.	10	4,940	58.7	8–121	1.19
	10 gal./acre ft.	10	4,410	184.8	64–722	4.19
	5 gal./acre ft.	7	9,700	1009.3	33–3,120	10.40

(b) *Recovery of worms from hosts exposed to affected (stage B) cercariae.*—The number of developing schistosomes recovered from hamsters exposed to cercariae reduced to stage B by treatment with the test emulsions was small compared to the number of cercariae used. In spite of this fact, it confirmed the belief that there is a possibility of infection by immobilized cercariae, and that the danger exists until the last cercaria is dead (Table 4).

Supplementary experiments showed that the number of affected cercariae penetrating a host was even greater if the cercariae were placed in a small container on the belly of the host. Schistosomes treated with DDT-xylene-Triton at the rates of 20, 10, and 5 gal./acre ft. yielded averages of 0.12, 0.24 and 1.33 per cent of the exposed cercarial population, respectively, as developing trematodes in the tissues of the hamsters (Table 4). Cercariae treated similarly with xylene-Triton emulsion concentrate yielded 0.13, 0.30 and 4.92 per cent, respectively, of developing worms in the hosts.

(c) *Recovery of parasites from hosts infected with revived cercariae.*—DDT-xylene-Triton emulsion concentrate applied at the rate of 20 gal./acre ft. stupefied and killed the majority of the cercariae under test by the end of two hours (Table 2 and Fig. 1). The total population of cercariae treated at this rate was, therefore, relatively inactive and often many of the animals became attached to the sides of the test vessels or lay on the bottom, appearing to be near death. With the replacing of most of the test water and its toxic emulsion with fresh water, a small percentage of the cercariae, within a few minutes, were revived sufficiently to infect the laboratory hosts, as shown in Table 4. The figures representing the number of flukes recovered were seemingly insignificant until compared with the number recovered from hamsters exposed to untreated cercariae (Table 4).

Hosts exposed to cercarial suspensions treated with smaller quantities of DDT-xylene-Triton yielded comparatively higher numbers of parasites. The maximum recovery of worms from one host so exposed was 14.72 per cent at 5 gal./acre ft. as compared to the average recovery of 13.30 from normal, untreated cercariae (Table 4).

Xylene-Triton emulsion minus DDT, when applied to schistosome cercariae at the rate of 20 gal./acre ft. showed considerable cercaricidal action (Table 4). As was expected, with the use of reduced rates of emulsion, there was an accompanying sharp rise in the percentage of recovery of parasites from exposed hamsters.

DISCUSSION

Data presented in this paper clearly indicate that DDT used in emulsions, in oil films, or in its crystalline form is not a dependable cercaricide at practical field rates of application. The fact that DDT does not immediately kill cercariae is not too surprising since other investigations (Richards, Cutkomp and Morton, 1944) have pointed out that animals with a chitinous cuticle show a marked susceptibility to DDT toxicity but non-chitinous animals do not. Cercariae are classed in the latter group. A recent report from this laboratory (Wilson, Kuntz and Green, 1945) has described preparations which are much more promising for the combined purposes of killing cercariae, mosquito larvae and snails.

In evaluating the toxicity of DDT it must be realized that tests were performed under laboratory conditions. Under such conditions the toxic agent exerts its

maximum effectiveness upon the cercariae and death is further accelerated by such factors as traumatic injury by handling and alteration of temperatures (Krakower, 1940, and Brady and Jones, 1945). Obviously, in the field, dosages as tested in these experiments would be less effective because of the influencing factors common in natural waters.

That DDT is not a reliable cercaricide is well borne out by the exposure experiments in which affected and revived schistosome cercariae were shown to be capable of infecting a host. Revival of treated cercariae in the present investigations, roughly simulated conditions in the field where treated cercariae in the upper layers of water could come into contact with fresh water at a depth below the effective range of the cercaricide, or could be swept by water currents into nearby untreated water. Although the percentage of recovery of worms from affected and revived cercariae was rather low, it represents a dangerous condition that could be magnified considerably in the field.

SUMMARY

1. DDT (dichloro-diphenyl-trichloroethane) powder, DDT in oils and in xylene-Triton emulsions, and Triton were tested to determine their effect on cercariae of *Schistosoma mansoni*. The results indicated that DDT alone or in any of the preparations tested was not sufficiently cercaricidal to be of practical value in the control of schistosomiasis.

2. If DDT is to be employed (in quantities recommended for mosquito control) for the mild cercaricidal action it possesses, its action is enhanced when it is incorporated in certain emulsions. For example, the emulsifying agent, Triton, itself incapacitated or immobilized the cercariae. A mixture of Triton and xylene is more toxic than Triton alone and the mixture is made still more lethal to the cercariae upon the addition of DDT. There is a tendency toward loss of toxicity of DDT-xylene-Triton and xylene-Triton emulsions upon standing or aging in open containers.

3. Cercariae, although severely affected and partially immobilized by DDT-xylene-Triton and xylene-Triton emulsion concentrates, are potentially infectious schistosomes until near death. A small but dangerous percentage of cercariae treated *in vitro* with the emulsions and placed in water will revive sufficiently to infect a host.

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POSSIBLE SNAIL HOSTS OF HUMAN SCHISTOSOMES IN THE UNITED STATES*

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The observations recorded in this paper constitute the final report to the Office of the Surgeon General of the United States Army on a three years' study to determine whether native snails of the United States may transmit the trematodes, *S. mansoni, S. haematobium,* and/or *S. japonicum,* which cause human schistosomiasis. The work was begun in July 1943 under a contract between the Office of Scientific Research and Development and New York University and continued under the Office of the Surgeon General after November 1st, 1945, when the contract was taken over by the Medical Department of the United States Army.

As a consequence of participation by the United States in the second world war, large numbers of military, naval, and civilian personnel were despatched through or operated in regions where schistosomiasis is endemic. The species of *Schistosoma* which cause the disease are well known and information concerning their distribution, incidence, life cycles, intermediate hosts, and bionomic relations is available in standard textbooks of parasitology and tropical medicine. A review of these data with a discussion of the clinical aspects, prognosis, and epidemiology of schistosomiasis was presented by Stunkard (1944) at the annual meeting of the New York State Association of Public Health Laboratories. More recent findings are reported in scientific journals, especially in the Bulletins of the Medical Departments of the United States Army and Navy. The realization that infection among American personnel was practically inescapable led to intensive research on methods of prophylaxis and treatment. Results of these studies, done by various groups, are now reaching publication. Preliminary reports by Hunt (1945) and Sullivan and Ferguson (1945) indicate the extent of infection in the armed forces.

But another aspect of the problem is presented by the return of infected personnel to the United States. Schistosomiasis is not easily diagnosed in the early stages and the only certain, positive criterion is the recovery of eggs of the parasites in the stools or urine of the patients (q.v., Thomas & Gage, 1945; Faust, Wright, McMullen, and Hunter, 1946; Nelson and Bayliss, 1946). Since 6–8 weeks intervene between exposure and the appearance of eggs in the dejecta of patients, prepatent infections could not have been identified on debarkation. Mild cases, in which few worms entered the skin (and it must be remembered that the parasites do not multiply in the human body), may manifest only slight and transitory symptoms and consequently may pass unnoticed. In such cases the eggs voided may be so few in number and passed at such irregular intervals that ordinary routine examination will fail to disclose them. Moreover, after treatment, serious cases of schistosomiasis may manifest an apparent recovery and eggs may be absent from the urine and/or stools for long periods of time, only to suffer relapses with the elimination of large num-

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bers of eggs. The return of servicemen has introduced and will continue to introduce the human schistosomes into the United States. The dispersal of these persons to all parts of the country implies that the infective larvae of the flukes will find their way in sewage to streams and water courses in diverse regions and under all sorts of climatic and ecological conditions. The presence in these ponds, lakes and streams of snails capable of serving as intermediate hosts would almost certainly lead to the establishment of the parasites and set up centers of infection. Medical literature records many instances in which parasitic diseases have been transplanted into new areas within historic time.

The human schistosomes, in different parts of the world, employ a great variety of snail hosts, members of a half dozen genera, distributed among three different families and two distinct zoological orders. Related species of snails are numerous in the continental United States and the possible establishment of schistosomiasis in North America is more than an academic postulate. Indeed, Cram, Jones, and Wright (1945) have already shown that *Tropicorbis havanensis,* a snail of the Southern United States, may transmit *S. mansoni,* the causative agent of human schistosomiasis in South America and the West Indies.

The schistosomes of birds are numerous and common throughout the United States and various native snails serve as their intermediate hosts. The cercariae of these bird blood flukes cause the peculiar "swimmers' itch" which has received much attention in recent years. McMullen and Beaver (1945) reported the life cycle of three of these species whose larval stages occur in the snails, *Stagnicola emarginata, Physa parkeri,* and *Lymnaea stagnalis.*

The only known schistosome of native wild mammals is *Schistosomatium douthitti* (Cort, 1914) Price, 1929 (= *S. pathlocopticum* Tanabe, 1923). Price (1931) described the life cycle and found the larval stages in *Lymnaea stagnalis, Physa gyrina,* and *Stagnicola palustris,* while the adult stage developed in wild mice, white mice, white rats, and the cat. Penner (1939) reported the species from the muskrat, guinea pig, wild rabbits, and the snowshoe hare. Penner (1941) exposed a young rhesus monkey to cercariae of *S. douthitti* and on autopsy 5½ days later found migrating worms " to be abundant in the lungs and slight hemorrhage was noted." He discussed the possibility of similar penetration and migration in man but his (1942) statement that cercariae of this species may cause "swimmers' itch" indicates that in the human host the larvae are held in the skin.

Cort (1918) discussed the adaptability of schistosome larvae to new hosts and pointed out that "the forked-tailed cercariae from the United States have shown several striking examples of lack of specificity in the choice of intermediate host. *Cercaria douthitti* Cort which was described (1915: 49) from *Lymnaea reflexa* Say taken near Chicago, Illinois, was later found in the region of Douglas Lake, Michigan, in *Lymnaea stagnalis oppressa* (Say), *Lymnaea stagnalis perampla* Walker, and *Physa ancillaria parkeri* (Cuvier)." He postulated that further studies will undoubtedly add to the list of snails that can transmit the human schistosomes. His paper, written almost thirty years ago, concludes with the following prescient paragraph: "Since the cercariae of the human schistosomes penetrate directly into their host, and can develop to maturity in rats, cats, dogs, and cattle, as well as man, they will probably spread rapidly if carried into any region where suitable intermediate hosts are found. It is known from case records and records of the immigration stations that *Schistosoma japonicum* has been brought into the United States from the

Orient. Before August, 1917, when schistosomiasis was placed on the exclusion list by the Surgeon-General of the United States Public Health Service, orientals with this disease are known to have entered this country in considerable numbers. In many of the irrigated regions of the Pacific Coast states, oriental laborers from countries in which schistosomiasis is prevalent live in much the same relation to the soil as in their own country, making ideal conditions for the spread of this disease provided a type of snail in which the flukes can develop is present. It is, therefore, evident that the question of the adaptability of the schistosomes to new intermediate hosts becomes a problem of great significance in relation to the possible spread of this disease in the United States, and it is of great importance to discover whether there are snails in this country in which the blood flukes can develop."

The present study was undertaken to determine whether or not native North American snails can serve as intermediate hosts and transmit the human schistosomes. To test these snails it was essential first to establish *Schistosoma mansoni, S. haematobium,* and *S. japonicum* in laboratory animals in order that miracidia of all three species would be constantly available for exposure of American snails. The snails that serve as natural intermediate hosts of the human schistosomes were maintained successfully throughout the study. Specimens were infected by miracidia from the vertebrate hosts and provided a continuous supply of cercariae. The cercariae were used to infect experimental animals which in turn provided the miracidia used for exposure of native snails.

MATERIAL AND METHODS

Schistosoma mansoni.—The strain of *S. mansoni* was obtained from Dr. Willard H. Wright of the National Institute of Health, Bethesda, Md., October 11th, 1943. Three specimens of *Australorbis glabratus* liberating cercariae of this species served as the source. This strain had been obtained originally from Puerto Rico and is the one endemic in the West Indies and South America. The snails multiplied rapidly, were easy to raise, and susceptible to infection by *S. mansoni* at any size from pinhead juveniles to fully mature adults. The stock has been maintained continuously in *A. glabratus* and in experimentally infected vertebrate hosts. Cercariae from the three snails were used to infect monkeys, Syrian hamsters, mice, and rabbits. The first monkey, a Javanese Cynomologus (*Pithecus* (*Macacus*) *mordax*) infected October 13th, 1943, negative to and on November 28th, began to pass eggs in the stools on November 30th, 1943, and another monkey, *Pithecus* (*M.*) *rhesus,* exposed on November 4th, 1943, last negative on January 26th, first passed eggs on January 28th, 1944. These monkeys are apparently healthy after carrying the infection since that time. The first monkey was infected by allowing it to pick bits of food from water containing a heavy suspension of cercariae, but the method is unsatisfactory since there is no precise information concerning the number of larvae which enter the skin. The second monkey was infected by 200–300 cercariae dropped slowly on the oral mucosa. The number of eggs passed by the monkeys increased for the first few weeks, remained relatively constant for a few months and gradually decreased thereafter. One of the monkeys was reinfected on September 24th, 1945.

It is currently believed that viable eggs of *Schistosoma mansoni* are voided only by man and monkeys and while other animals can be infected, that they do not pass eggs of the parasite and are not concerned in transmission of the disease. Our experience in general supports this belief. Rabbits, rats, cats, guinea pigs, and

hamsters were infected, but ordinarily no viable eggs were recovered in the feces. Brumpt (1930) reported six generations of *S. mansoni* in mice; he obtained miracidia for infection of snails, *Planorbis boissyi,* by recovering eggs from livers of the mice. In our laboratory, heavily infected mice lived about eight weeks and died, often rather suddenly, from an apparent toxemia. A mouse infected in November 1943 died in January 1944; on autopsy, the rectum was full of blood. This material was washed into a watch glass and found to contain a large number of *S. mansoni* eggs. The eggs were washed and contained active miracidia, which hatched in the course of a few hours. These miracidia were used to infect *A. glabratus.* Cercariae from these snails were used to infect mice, hamsters, and other rodents. From them, we have developed a strain of *S. mansoni* in mice and hamsters which was carried through ten successive generations. After the fourth generation, the mice and hamsters regularly passed viable eggs in the stools and this mouse-hamster strain has been used extensively in our later experimental work. Guinea pigs, infected with cercariae of the eighth generation, also passed viable eggs regularly in the stools. This rodent strain developed well in Syrian hamsters and in mice, but we had less satisfactory results with rats and rabbits. The worms developed to maturity in rats and rabbits but viable eggs were not recovered in the feces.

The intermediate host of *S. mansoni* in Egypt, *Planorbis boissyi,* was supplied by Dr. Claude H. Barlow, Bilharzia Section, Ministry of Public Health, Cairo, Egypt. One-hundred eighty-two specimens collected near Cairo January 31st, 1944, were sent by air mail and received the 8th of February, 1944. Eighty snails survived and although a few cercariae were shed, the Egyptian strain of *S. mansoni* was not established in laboratory animals. Dr. Barlow arrived in the United States July 7th, 1944, bringing additional specimens of *P. boissyi,* but none of the infected individuals survived. This species has been maintained in the laboratory since its reception February 8th, 1944, and although hundreds of individuals have been exposed to miracidia of the West Indian strain of *S. mansoni,* no cercariae have been obtained. The miracidia attacked and penetrated the snails, but development was never completed and snails dissected 10–12 weeks after exposure showed no trace of the parasite.

After Cram, Jones, and Wright (1945) demonstrated experimentally that *Tropicorbis havanensis* could serve as an intermediate host and transmit *S. mansoni,* attempts were made to repeat the experiment and confirm their results. From six specimens of *T. havanensis* provided by the National Institute of Health, we raised a stock of these snails. During a period of over a year, although hundreds of specimens were exposed to miracidia of the West Indian strain of *S. mansoni,* we never obtained cercariae. As in the case of *P. boissyi,* the miracidia penetrated the snails, transformed into primary sporocysts but there was little or no further development. Mature daughter sporocysts were never obtained and the parasites eventually were resorbed.

Schistosoma haematobium.—Material of this species was supplied by Dr. C. H. Barlow. Four-hundred twenty-six specimens of *Bulinus truncatus* collected near Cairo January 31st, 1944, were part of a shipment which arrived by airplane on February 8th. One-hundred fifty-six snails survived but none of them was infected. These specimens laid eggs and the species has been maintained continuously in the laboratory. It appears probable that the infected individuals are less hardy and succumbed in transit. Dr. Barlow arrived in New York on July 7th, 1944, with

additional specimens of *Bulinus truncatus* which were turned over to the National Institute of Health, and a baboon which had been infected June 30th, 1944. The animal began to pass eggs in both feces and urine on August 25th, 1944. Miracidia from these eggs were used to infect *Bulinus truncatus* and the resulting cercariae were employed for the infection of other laboratory animals. *S. haematobium* was maintained continuously throughout the study. Dr. Barlow also brought a hedgehog and two fat-tailed jerboas, *Pachyuromys duprasi,* which had been infected with *S. haematobium.* The hedgehog died four days after arrival, one jerboa was sent to the National Institute of Health, the other passed eggs in the feces but the eggs were small, irregular in shape and the larvae were dead. The animal died on October 9th and at autopsy 8 pairs of worms and an additional single male were found in the large mesenteric vein. The worms were very small, about one-third the length of specimens from man, with delicate musculature and internal organs. The baboon died on the 30th of January, 1945. The animal was in apparent good health on the previous evening at 6:00 o'clock. He had eaten unusually well that day. At 9:00 o'clock the next morning he was lying on the floor of the cage unable to raise his head. He died at 11:00 o'clock and was autopsied almost immediately thereafter by Dr. H. E. Meleney of the New York University Medical School, New York City. It appeared that the baboon infected with *S. haematobium,* like the mice infected with *S. mansoni,* succumbed suddenly when the infection had reached a particular stage. Eggs of *S. haematobium* passed by the baboon, diminished gradually in number and few were being liberated at the time of his death. After the first baboon died, a monkey and subsequently two other baboons were infected with cercariae of *S. haematobium.* They have continued to pass eggs for well over a year although the number of eggs has declined.

Attempts were made to infect other laboratory animals but viable eggs were not passed by them, and we were unable to establish a rodent strain of *S. haematobium.* Brumpt (1928) apparently was the first to complete the cycle from egg to egg in lower animals, recovering the eggs from the liver of a mouse. We were able to confirm his report, but the method is not suitable for our experiment since eggs from baboons and a monkey were always available.

In addition to *Bulinus truncatus,* which serves as the intermediate host of *S. haematobium* in Egypt, we received from Dr. R. E. Kuntz of the U. S. Naval Medical Research Institute, Bethesda, Md., specimens of *Physopsis africana,* which serves as the intermediate host of this species in Western and Southern Africa. These snails lived well in the laboratory; eggs and young were obtained. Specimens were exposed to miracidia of *S. haematobium* but had not yet begun to liberate cercariae at the conclusion of the experiment.

Schistosoma japonicum.—Our stock of *S. japonicum* was derived from specimens of *Schistosomophora quadrasi* collected in Leyte and supplied by the National Institute of Health, January 30th, 1945, and April 9th, 1945. These snails liberated a few cercariae and provided larvae for the infection of experimental animals. Later, June 28th, 1945, Dr. Wright sent a heavily infected dog which passed eggs in large numbers. Subsequently, he sent additional specimens of *S. quadrasi.* Specimens of *Katayama nosophora* were supplied by Major F. B. Bang through the good offices of Dr. Meleney and also by Dr. R. E. Kuntz. Both species have been maintained in the laboratory and young of both species have been raised. These snails are difficult to maintain. Since they are amphibious and will not stay in the water and since

they will not eat the lettuce or the algae ordinarily provided as food for other snails, special arrangements were provided for them. Aquaria were prepared with mud at one end, only partially covered by water and provided with bits of decomposing leaves. The leaves were streaked or smeared lightly with yeast and diatoms were raised and added to supplement the food. Although acid water was prepared by soaking dead leaves in distilled water, and aquaria of various hydrogen-ion concentrations from pH 6 to pH 7 were tried, the snails lived and reproduced as well in ordinary spring water with a pH of 7.3 to 7.7 as in the aquaria in which organic acids leached from dead leaves were added to lower the pH. The naturally infected snails and those infected in the laboratory shed cercariae at irregular and infrequent intervals. The most effective method of obtaining cercariae was to keep the snails for several days on moist earth and leaves and then put them in water. Our experience suggests that in nature the cercariae are liberated after rains.

Collection of miracidia and exposure of snails.—Eggs containing miracidia were obtained from monkeys, baboons, mice, and hamsters, maintained in the laboratory for that purpose. The same procedure was followed in exposing both the snails which serve as natural hosts of the schistosomes and the native North American snails which were tested to determine whether or not they could transmit the parasites. Eggs of *S. haematobium,* recovered from urine, began to hatch in a few minutes when placed in spring water. Eggs of *S. haematobium,* recovered from feces, did not hatch so soon and were handled by the same method as those of *S. mansoni* and *S. japonicum* which are voided only in feces. The fecal material was broken up in a beaker of water and strained through a fine-meshed wire screen. The eggs were allowed to settle and the water decanted. The washing process was repeated until the supernatant fluid remained clear. The sediment was then transferred to Erlenmeyer flasks which were filled with warm spring water, 70°–80° F. Eggs of *S. haematobium* began to hatch in 2–3 hours, whereas those of *S. mansoni* and *S. japonicum* required 12–48 hours. The liberated miracidia accumulated in the neck of the flask where they could be observed by holding the flask toward the light. This hatching technique is the surest and best method of determining the presence of viable eggs in stools. Microscopic examination of feces, even with the aid of concentration techniques, is tedious, slow, and if only a few eggs are present they may be entirely overlooked. When there are only a half dozen viable eggs in a stool sample, it would be virtually impossible to find them by microscopic examination, whereas after hatching, the swimming larvae may be readily recovered from the neck of the flask.

In certain experiments the washed fecal material was added to aquaria in which the snails were maintained. It was believed that such mass exposure might more nearly simulate conditions which obtain in nature. As a rule, however, individual snails were exposed in stender dishes to three or four miracidia for 24 hours. The miracidia were removed by pipette from the hatching flasks to the dishes in which snails were exposed. With this method it is possible to observe the snail, to determine whether or not the miracidia are attracted to it, and to watch the swimming movements, attachment, and penetration of the larvae. After 24 hours of exposure, the snails were transferred to finger bowls, or larger aquaria, where they were maintained for observation. Each stender dish was then examined for miracidia which had failed to penetrate. Since the purpose of the study was to determine whether or

not native snails could serve as intermediate hosts and transmit the parasites, snails were not killed so long as they remained in good condition. In many instances they were exposed a second and in a few instances a third time. It was only natural that some of the snails died shortly after exposure and others after longer periods. Dead snails decompose very rapidly and after a few hours the tissues were so decayed that little or nothing could be learned concerning an infection. Moribund snails were sometimes killed and these specimens and others examined immediately after death, disclosed the status of any infection present. After removal of the shell, small specimens were pressed between glass slides and examined microscopically. Larger specimens were dissected under a binocular microscope and the separate portions were examined between glass slides.

As noted previously, exposures were made both individually and in mass. Since conditions in the laboratory seldom if ever entirely duplicate those in nature, the conditions of exposure were varied as much as possible. Spring water was used and adjusted to various hydrogen-ion concentrations from pH 6.5 to 7.8. Exposures were made at different light intensities, different temperatures, and re-exposures were made at different intervals. Since young snails are often more susceptible to trematode infections, individuals of various sizes were selected. Before and after exposure, the snails were maintained under ordinary room conditions with fluctuating light and temperature.

In order to make the study really comprehensive, efforts were made to obtain as many species of native snails as possible. Collections were made in different localities, where different geological formations and ecological habitats influence the number and type of snails. The writer made collections in New England, New York, New Jersey, and Pennsylvania. These collections were supplemented by the purchase of additional species from commercial establishments including the General Biological Supply House in Chicago, Lakeview Ponds in Cincinnati, the Carolina Biological Supply Co., Elon College, North Carolina, the Southern Biological Supply Co. in New Orleans, and other sources. Additional specimens were received from personal friends, from present and former students of New York University, and in response to a request kindly published in the Turtox News of November, 1943. During the summer of 1945, Dr. Henry van der Schalie of the University of Michigan made extensive collections, especially of amnicolid snails, within a radius of 300 miles of Ann Arbor, Mich.

On collection or receipt, the snails were identified or representative specimens were killed and fixed for subsequent identification by experts. Dr. van der Schalie identified the snails sent by him and many of the others were determined by Dr. H. A. Pilsbry of the Academy of Natural Sciences, Philadelphia, Pa. To all those who contributed material for the study, grateful acknowledgment is made.

Representatives of each species, and of the same species from different localities, were installed in separate aquaria, in attempts to establish breeding colonies from which laboratory-raised specimens could be obtained later for exposure to miracidia. Collections could not be made during the colder months and consequently it was essential to have laboratory-raised snails for the work in winter. Since different species of snails manifest different requirements or preferences in food, in the chemical composition and hydrogen-ion concentration of water, and in various other environmental factors, the maintenance of these many species required the use of a large

number of aquaria. The results were not uniformly happy, since it is very difficult to duplicate natural conditions in the laboratory, the requirements of many species are largely unknown, and several species are exceedingly difficult to maintain. It was important, however, in so far as possible to have laboratory-raised specimens for exposure, since ordinarily juvenile snails are more susceptible to trematode infection. Moreover, the use of such specimens provided the best measure of control, since they were known to be free from any previously acquired infection.

After the specimens selected for breeding colonies had been installed in aquaria, the remaining wild snails were kept in finger bowls or small aquaria until exposed to miracidia. Attempts were made to supply them with the type of vegetation on which they naturally live, but mortality was often heavy. Many of the snails that had been shipped for long distances were dead or moribund on arrival, and others, that were in poor condition, did not long survive.

During the first year the study was limited to work on *S. mansoni,* as it was the only species available at the time. Attention was directed primarily to planorbid snails, since members of this family transmit the parasite in Africa, South America, and the West Indies. Receipt of *S. haematobium* in the summer of 1944 permitted exposure of snails to miracidia of this species as well as those of *S. mansoni.* Lymnaeid snails, especially, were tested as possible intermediate hosts of *S. haematobium. Schistosoma japonicum* became available in the summer of 1945 and during the last year efforts were largely devoted to work with this species. Amnicolid snails, particularly, were tested as possible intermediate hosts, since members of this family transmit the parasite in the Far East.

MATERIAL SUPPLIED TO OTHER INSTITUTIONS

During the course of this study, with approval from the Office of the Surgeon General, we have supplied strains of the human schistosomes to other laboratories. These include the New York University Medical School, the Columbia University Medical School and the Cornell University Medical School, all in New York City; the Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.; the Harvard University Graduate School of Engineering, Cambridge, Mass.; the Western Reserve University Medical School, Cleveland, Ohio; the University of Minnesota Medical School, Minneapolis, Minn.; Tulane University Medical School, New Orleans, La.; the American Cyanamid Co., Stamford, Conn.; the Lederle Laboratories, Pearl River, N. Y.; the Rockefeller Institute for Medical Research, Princeton, N. J.; the National Institute of Health and the United States Naval Medical Research Institute, both at Bethesda, Md.; and the Medical Research Council of Great Britain (National Institute for Medical Research), Hampstead, London.

To laboratories engaged under OSRD or other government contracts in testing chemical compounds for possible therapeutic value against schistosomiasis; the Western Reserve University Medical School, the University of Minnesota Medical School, and the Johns Hopkins University School of Hygiene and Public Health; we have provided 30 rabbits, 100 hamsters and 14,576 mice infected with *S. mansoni.* For infection of vertebrate hosts, we have placed them in water containing cercariae or injected the cercariae either subcutaneously or intraperitoneally. The animals may be infected by any of the three methods, but all the mice supplied for therapeutic studies were injected.

At the conclusion of the study, our infected animals including the stocks of *Australorbis glabratus, Planorbis boissyi, Tropicorbis havanensis, Bulinus truncatus, Physopsis africana, Schistosomorpha quadrasi,* and *Katayama nosophora,* were divided between Dr. W. H. Wright, National Institute of Health, and Dr. H. E. Meleney, New York University Medical School.

RESULTS OF ATTEMPTS TO INFECT NORTH AMERICAN SNAILS

Representatives of the more common and abundant snails were exposed to miracidia of the three species of human schistosomes. In view of the many and varied sources from which these snails were obtained, precise localities are available only for those collected by the writer. The region where specimens were collected probably has no relation to susceptibility to infection, although different genetic strains of a single species from the same locality may manifest varying degrees of resistance, e.g., the results with *Tropicorbis havenensis.* The miracidia were attracted in greater or lesser degree to several species of snails, and even in the absence of definite attraction, the larvae attacked and penetrated into the majority of species

TABLE 1.—*North American snails exposed to miracidia of* S. mansoni

Species	Locality	Notations
Helisoma trivolvis	Ohio, Ill., N. Car., N. Y., Conn., Mass.	(72) mostly LR; primary sporocysts containing germ balls found in 2 LR specimens, Illinois strain.
Helisoma anceps	N. Y., N. J., Penn.	(42) mostly LR; penetration, no development.
Helisoma lentum	La., Fla.	(35) all LR; penetration, no development.
Helisoma duryi	La., Fla.	(19) 10 LR; penetration, no development.
Gyraulus parvus	Mass., N. Y., N. J., Penn.	(81) less than half LR; penetration, no development.
Gyraulus hirsutus	Mich.	(11) all FC; penetration, no development.
Menetus exacuous	N. Y., N. J., Penn.	(15) 8 LR; penetration, no development.
Tropicorbis havanensis	La., from Nat. Inst. Health strain	(34) all LR; penetration, no development.
Physa heterostropha	N. Y., N. J., Conn., Mass.	(32) mostly LR; penetration, in a LR specimen found sporocyst with small germ balls.
Physa integra	N. Y., Penn.	(5) all FC; penetration doubtful, no sporocysts found.
Physa gyrina	Ill.	(8) all FC; penetration doubtful, no sporocysts found.
Physa oleacea	La.	(10) 6 LR; penetration doubtful, no sporocysts found.
Physa anatina	N. Mex.	(6) all FC; penetration not observed, no sporocysts found.
Lymnaea palustris	Mass., N. Y., Mich.	(53) 20 LR; penetration doubtful, no sporocysts found.
Lymnaea humilis	N. Y., Penn., Mich., Ill.	(8) all FC; penetration doubtful, no sporocysts found.
Pseudosuccinea columella	N. Y.	(12) 8 LR; penetration, no development.
Lyogyrus granum	N. Y., Conn., Penn.	(16) 6 LR; penetration doubtful, no sporocysts found.
Pleurocera subulare	Mo.	(6) all FC; penetration doubtful, no sporocysts found.
Goniobasis plebeius	Mo.	(5) all FC; penetration doubtful, no sporocysts found.
Viviparus malleatus	Ohio	(26) mostly LR; penetration, primary sporocysts with germ balls found in 2 snails.
Neretina reclivata	Fla., La.	(8) all LR; penetration, no sporocysts found.
Pomacea sp.	Fla., N. Car.	(5) all LR; penetration doubtful, no sporocysts found.

tested. Often the larvae would swarm about a snail, attach, and later swim away. Observation of penetration was difficult, as a larva would attack under the shell or at a point which was hard to keep in the field of the microscope and movements of the snail interfered with constant observation. If a miracidium entered a snail and reached the haemocoele, usually the cilia were lost and it transformed into a sporocyst. In a few instances the germinal cells in the sporocyst began to multiply, but development was very slow and soon stopped entirely. Apparently the parasites died and were absorbed, since in snails dissected a month or two after exposure there were no traces of sporocysts. Of the total number of snails exposed, only a small percentage were examined microscopically. In no case were cercariae obtained. Snails collected in the field sometimes harbored larval stages of other trematodes, but in no instance did the previous natural infection appear to influence either the behavior of miracidia or their development in case they entered the snail.

TABLE 2.—*North American snails exposed to miracidia of* S. haematobium

Species	Locality	Notations
Lymnaea palustris	Ohio, Mich.	(61) 21 LR; penetration, no development.
Lymnaea obrussa	Mich.	(10) all FC; penetration doubtful, no sporocysts found.
Lymnaea humilis	N. Y., Penn., Mich., Ill.	(6) all FC; penetration doubtful, no sporocysts found.
Lymnaea sp. unidentified amphibious species	Mich.	(15) all FC; penetration doubtful, no sporocysts found.
Pseudosuccinea columella	N. Y.	(16) 10 LR; penetration, no development.
Physa heterostropha	Vt., Mass., N. Y., N. J., Penn.	(32) all LR; penetration, no development.
Physa gyrina	Ill., Mich.	(8) all FC; penetration doubtful, no sporocysts found.
Physa anatina	N. Mex.	(12) 8 LR; penetration doubtful, no sporocysts found.
Physa oleacea	La.	(14) 4 LR; penetration doubtful, no sporocysts found.
Physa integra	N. Y., Penn.	(12) all FC; penetration doubtful, no sporocysts found.
Bythinia tentaculata	Mich.	(35) all FC; penetration, no development.
Goniobasis livescens	Mo.	(22) all FC; penetration doubtful, no sporocysts found.
Pleurocera acuta	Mich.	(14) all FC; penetration doubtful, no sporocysts found.
Helisoma trivolvis	N. Car., Ill., Ohio, N. Y., Mass.	(34) all LR; penetration, no development.
Helisoma duryi	Fla.	(6) all LR; penetration doubtful, no sporocysts found.
Helisoma anceps	Mass., N. Y.	(16) all LR; penetration, no development.
Gyraulus parvus	N. Y.	(18) 10 LR; penetration, no development.
Tropicorbis havanensis	La.	(14) all LR; penetration, no development.
Pomatiopsis lapidaria	Mich.	(42) all FC; penetration, no development.
Lyogyrus granum	N. Y., Pa., Conn.	(6) all FC; penetration doubtful, no sporocysts found.
Amnicola walkeri	Mich.	(10) all FC; penetration doubtful, no sporocysts found.
Viviparus malleatus	Ohio	(6) all LR; penetration doubtful, no sporocysts found.
Pomacea sp.	Fla., N. Car.	(8) all LR; penetration doubtful, no sporocysts found.
Succinea avara	Mich.	(22) all FC; no penetration observed.
Succinea retusa	Mich.	(26) all FC; no penetration observed.

Since the results were consistently negative, they are presented in abbreviated form in the accompanying tables. Locality denotes the state where the snails or their progenitors were collected. In each instance, the figure in parenthesis refers to the number of specimens examined microscopically. Examinations extended over a period from a few hours to several weeks after exposure of the snails to miracidia. Dates of exposure and examination are available but probably are not significant. The letters FC, signify field collected; LR, laboratory raised specimens.

TABLE 3.—*North American snails exposed to miracidia of* S. japonicum

Species	Locality	Notations
Pomatiopsis lapidaria	Mich.	(376) all FC; penetration, results equivocal, see discussion.
Pomatiopsis cincinnatiensis	Mich.	(120) all FC; penetration, no development.
Bythinia tentaculata	Mich.	(35) all FC; penetration, no development.
Lyogyrus granum	N. Y., Penn.	(275) 68 LR; penetration, no development.
Amnicola lustrica	Mich.	(12) all FC; penetration, no development.
Amnicola integra	Mich.	(16) all FC; penetration, no development.
Amnicola walkeri	Mich.	(34) all FC; penetration, no development.
Amnicola limosa	Mich.	(36) all FC; penetration, no development.
Hydrobia (Paludestrina) nickliniana	Mich., N. Y.	(10) all FC; penetration doubtful, no sporocysts found.
Lymnaea palustris	N. Y., Mich.	(53) 20 LR; penetration, no development.
Lymnaea obrussa	Mich.	(10) all FC; penetration doubtful, no sporocysts found.
Lymnaea sp. amphibious species	Mich.	(10) all FC; penetration doubtful, no sporocysts found.
Helisoma trivolvis	Mass., N. Y., N. J.	(26) all LR; penetration, no development.
Helisoma anceps	N. Y., Penn.	(13) all LR; penetration, no development.
Helisoma lentum	La.	(11) all LR; penetration, no development.
Tropicorbis havanensis	La.	(10) all LR; penetration, no development.
Pleurocera acuta	Mich.	(19) all FC; penetration doubtful, no sporocysts found.
Goniobasis livescens	Mich.	(11) all FC; penetration doubtful, no sporocysts found.
Aplexa hypnorum	Mich.	(12) all FC; penetration doubtful, no sporocysts found.
Campeloma sp.	N. Y.	(12) all FC; no penetration.
Neretina reclivata	Fla.	(14) all LR; penetration doubtful, no sporocysts found.
Viviparus malleatus	Fla.	(12) all LR; penetration doubtful, no sporocysts found.
Succinea avara	Mich.	(10) all FC; no penetration.
Succinea retusa	Mich.	(10) all FC; no penetration.

DISCUSSION

Representatives of the more common and readily available snails from Eastern United States have been exposed to miracidia of the human schistosomes with negative results. Twenty-two species were tested with miracidia of *S. mansoni,* twenty-five species with miracidia of *S. haematobium,* and twenty-five species with miracidia of *S. japonicum.* The failure to obtain cercariae was not surprising, since it is often difficult to consummate well-known trematode life cycles under laboratory conditions, even when natural intermediate hosts are employed.

Since Cram, Jones, and Wright (1945) had completed the life cycle of *S. mansoni* in *Tropicorbis havanensis,* repeated attempts were made under various conditions to infect members of this species. As stated earlier, our stock of snails were descendents of specimens from their colony, and our failure to duplicate their success is not explained. The snails reproduced well and appeared in good condition; although miracidia penetrated and transformed into sporocysts, there was little or no development. It is clear, therefore, that failure to infect a sample of any species does not thereby demonstrate that the species is incapable of transmitting the parasite. Although the specimens tested may prove refractory, other individuals of the same species may be suitable intermediate hosts and permit development to continue with liberation of infective cercariae. Accordingly, the negative results reported in this paper are inconclusive, and indeed they might easily lead to a false sense of security.

Of the three human schistosomes, the establishment of *S. japonicum* in the United States would constitute the most serious danger. Since these parasites can develop in wild and domestic mammals as well as in man, and since these animals pass viable eggs of the flukes, if a suitable snail vector were present in an area and an infection were started, it could be maintained in the absence of human cases. The introduction of oncomelanid snails could provide such favorable intermediate hosts and care should be exercised to prevent the entrance of these species into the United States.

Since amnicolid snails serve as intermediate hosts of *S. japonicum* in the Far East, special efforts were made to test North American members of this family. Attention was directed particularly to species of *Pomatiopsis,* since in morphology, habits, and ecological relations they resemble the natural hosts of *S. japonicum. Pomatiopsis cincinnatiensis* did not survive well under laboratory conditions; specimens of *P. lapidaria,* however, remained alive for many weeks, although reproduction was not obtained. Seven specimens of *P. lapidaria* were exposed to miracidia of *S. japonicum* on September 3rd, 1945; one died October 23rd and contained daughter sporocysts but the cercariae were too immature to be identified. Three of these snails died on October 26th and two of them contained sporocysts similar to those obtained three days earlier. One snail died on October 27th and another on October 30th. Both contained the same kind of sporocysts. The seventh snail died on November 1st and was not infected. None of these snails shed cercariae and on dissection, the infections they carried were too immature to be positively identified. Young cercariae were present in the sporocysts, but they were so immature that it was impossible to tell whether they had bifurcate tails. Since eight weeks had elapsed since exposure, and a longer time since the snails were collected and could have acquired a natural infection, and since the cercariae were not yet sufficiently developed to permit positive identification, it appeared probable that the infections were experimental ones. Forty-one specimens of *P. lapidaria* received in the same shipment, some of them moribund, had been dissected and none of them contained larval trematodes, which supported the belief that the infection was actually *S. japonicum.* Since that time, two series of exposures have been made, one in the late fall of 1945 and the other in the spring of 1946. In neither case were experimental infections established and consequently the nature of the one found in October, 1945 remains obscure. Natural infections with other trematodes, particularly a virgulate cercaria, were found in *P. lapidaria.*

It should be recognized that in the present study only a very meager sample of the total snail species in North America has been tested. Moreover, in each species, the number of individuals exposed is actually very small. It is well known, and the experience with *Tropicorbis havanensis* is a good example, that when a trematode species is introduced to a new snail host, the great majority of the snails are refractory. Very little is known concerning the factors which determine or affect susceptibility and resistance. The relationships between host and parasite rest on a chemical basis and are relative; they may be influenced by the physiological conditions of either or both host and parasite. In certain instances, refractory individuals may be rendered susceptible if maintained on a deficient diet. Other factors are undoubtedly concerned. One of them appears to be genetic, since within a single species certain individuals and their progeny are susceptible whereas other strains are consistently refractory. Even among susceptible stocks, certain individuals are more resistant than others. This fact is a truism of medicine, and apparently applies to infection of lower animals as well.

All animals manifest variations, especially physiological changes, which may be correlated with age, sexual maturity, nutritional state, and various other factors. Probably no two individuals are exactly alike. Living organisms are not only variable but plastic; changes in environment and ecological relations may induce modifications and physiological adjustments in the organisms themselves. Such adaptive responses are too well known to require discussion or verification. These biological readjustments are concerned in the acquisition of new hosts by parasitic worms. Many trematode species have changed their hosts from time to time and the introduction of a species into a new region has often resulted in its establishment in a new host species. One of the most conspicuous examples is the sheep liver fluke, *Fasciola hepatica,* although the human schistosomes manifest the same phenomenon. In different regions of the Far East, different species of oncomelanid snails serve as natural hosts of *S. japonicum,* although in any particular region a single species of snail is the preferred host of the parasite. *Schistosoma haematobium* is transmitted in Egypt by *Bulinus truncatus,* in central and southern Africa the principal host is *Physopsis africana,* while in Morocco and Portugal the preferred host is *Planorbis dufourii. Schistosoma mansoni* in Egypt is transmitted by *Planorbis boissyi,* although in other regions of Africa other species of *Planorbis* are the preferred hosts. The parasite has been reported also from *Physopsis africana* and *Bulinus tropicus.* It is generally believed that *S. mansoni* was introduced into South America and the West Indies with African slaves and in the Western Hemisphere it utilizes *Australorbis glabratus* as the intermediate host. It is interesting to note that repeated attempts to infect *Planorbis boissyi* with miracidia of the West Indian strain of *S. mansoni* have yielded consistently negative results. The larvae penetrated the snails but development did not proceed any better in *P. boissyi* than in native North American snails. Once adapted to a particular host species, the parasite will select that species in preference to the one previously employed. The adaptation of living organisms to new environments may be gradual and progressive. I believe it probable that if *S. mansoni* is maintained for a considerable time in susceptible strains of *Tropicorbis havanensis* that the parasite will become adjusted to that species and able to complete its development in strains that are now refractory. Adoption by trematode parasites of new intermediate hosts may be paralleled by adaptation to new vertebrate

hosts. The selection and development of a strain of *S. mansoni* which after a few generations in rodents was so altered that viable eggs were passed regularly by these animals (p. 542) further indicates the plasticity of the schistosomes.

The acquisition of new intermediate hosts by digenetic trematodes in general and schistosomes in particular indicates strongly that if large numbers of eggs for a considerable period of time are passed into the water courses of any region, the parasite may eventually find a susceptible host and become established there. The results of the present study, however, indicate little likelihood that the human schistosomes will become established in the United States. The number of infected persons in the country is small, the number of eggs voided will not be great, the larvae are short-lived and the probability of their finding a susceptible snail is so remote that there is slight ground for apprehension. Furthermore, as noted by Stoll (1943), no autochthonous cases of schistosomiasis have been reported from either France or the United States despite the presence of endemic foci in adjacent areas and numerous importations of infected persons, with sanitary practices that would favor the establishment of the parasites. For more than 100 years such persons from the Mediterranean area have entered France and during a similar period the importation of African slaves must have brought both *S. haematobium* and *S. mansoni* to North America. The entry of Chinese and Japanese, principally on the West Coast, has introduced *S. japonicum* into agricultural areas of that region. These extensive and long continued importations constitute natural experiments of extraordinary scope and the failure hitherto of the parasites to become established in the United States is a significant and heartening epidemiological fact.

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THE LIFE HISTORY OF *PLAGITURA SALAMANDRA* HOLL, 1928 (TREMATODA: PLAGIORCHIIDAE)

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I. INTRODUCTION

This work was undertaken with the idea of throwing some light on the validity of the two species of *Plagitura, P. salamandra* Holl, 1928, and *P. parva* Stunkard, 1933, occurring in the intestine of the newt, *Triturus viridescens.*

The genus, *Plagitura,* was erected by Holl (1928) to contain a species of trematode found by him near Durham, North Carolina, and which he called *Plagitura salamandra.* Stunkard (1933a, 1933b) reported on the morphology and preliminary work on the life cycle of a new species, *Plagitura parva,* collected from various points on the eastern coast of the United States. T. D. Brown in 1934 (unpublished thesis, University of Virginia) arrived at the same conclusion as Price (1930) had, *viz.,* that *P. salamandra* Holl, 1928, was a synonym of *Manodistomum occultum* Stafford, 1905. Kelley (1934) recognized the validity of *Plagitura salamandra* and reported on the great variation in *P. salamandra* taken from newts collected from Massachusetts, Pennsylvania, Ohio, and Virginia. Stunkard (1936) reported the complete life cycle of *P. parva* and contended that by the recognition of two species the variations reported by Kelley could be explained. Furthermore, he recognized the validity of *Manodistomum occultum* and *Plagitura salamandra.*

In his life history studies, Stunkard found that the eggs of *P. parva* were ingested by the snail, *Helisoma anceps* (Menke) = *H. antrosa* (Conrad) *in sensu* Stunkard (1936) where they hatched. The miracidia penetrate the gut wall and develop into sporocysts. There is a second generation of sporocysts which in turn produce cercariae. The cercariae are provided with stylets and proteolytic glands with which they penetrate several species of snails and aquatic insect larvae where they encyst as metacercariae. The final host, *Triturus viridescens,* becomes infected with the adults as a result of ingesting the encysted metacercariae.

Stunkard (1936) reported that *Helisoma anceps* failed to become infected when fed eggs of *Plagitura salamandra.*

The snail *Pseudosuccinea columella* (Say) is abundant in the vicinity of Charlottesville, Virginia. I. M. Schor in 1934 (unpublished thesis, University of Virginia) recorded many observations on the cercariae escaping from this snail as well as the fact that these cercariae would not encyst in tadpoles. When it was found that the cercaria from *Pseudosuccinea columella* was morphologically similar to the cercaria of *Plagitura parva,* as reported by Stunkard (1936), experiments were undertaken to see if the life cycle of this species could be experimentally worked out. A preliminary report of these experiments was made at the 1943 meeting of the Virginia Academy of Science (Owen, 1943).

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II. MATERIALS AND METHODS

Living materials, collected as needed from ponds and streams in the vicinity of the biological laboratory, Charlottesville, Virginia, were used throughout this study. Concurrently with the study of living material, infected snails and insect larvae were fixed for the preparation of permanent mounts. Both whole mounts and sectioned material of stages in the life history were made and used for additional checks on measurements and observations.

Mature eggs of *P. salamandra,* removed by a capillary pipette from newt feces, were placed in pond water for at least four days. A minute piece of washed lettuce, bearing eggs, was placed in each of several Syracuse watch glasses containing boiled oxygenated tap water. The laboratory-reared snails were washed in tap water and placed on the piece of lettuce and allowed to remain until it was certain that ingestion of eggs had taken place. The snails were removed, washed, and then dissected at intervals of one-half hour, one hour, two hours, four hours, six hours, twelve hours, twenty-four hours, forty-eight hours, one week, and two weeks. The intestine, in each case, was then placed in a separate watch glass and examined under the compound microscope. As a check on the place and time of hatching in the molluscan host and the possible migration of the miracidia and the development of sporocysts, an equal number of similarly infected snails were fixed in warm Bouin's; after their shells were removed, they were serially sectioned and stained with Delafield's haematoxylin.

The processes of infection and development of the fluke in the second intermediate host were determined by exposing *P. columella, Helisoma anceps,* and aquatic insect larvae to actively swimming cercariae. Dissections and fixations of the exposed organisms were made at the end of eighteen hours, thirty-eight hours, and one week.

Other workers in this laboratory have found that newts lose their trematode infections when kept in aquaria for two or more months; therefore, the four newts used in these feeding experiments with experimentally infected second intermediate hosts were isolated in the laboratory for four months. Three of the newts were put into separate butter dishes and fed dragonfly larvae and snails which had previously been exposed to cercariae. Two of the newts were dissected at the end of two days. One was dissected at the end of eleven and one-half days. One was dissected as a control.

III. EXPERIMENTAL RESULTS AND OBSERVATIONS

A. Hatching of Eggs

Eggs of *Plagitura salamandra* were fed to laboratory-raised specimens of *Pseudosuccinea columella* and *Helisoma anceps* and to individuals of the same species collected in the field. The snails had been isolated for forty-eight hours.

To determine the time when eggs become infective, ten uninfected snails of *P. columella* and *H. anceps* each were fed eggs in the following manner: (1) five with eggs taken from the feces of the newt and allowed to stand in pond water for four days; (2) five with eggs taken from recently passed feces. The miracidia are not active or infective when the eggs are passed in the feces of the newt. The miracidia become active and infective after standing in pond water at room temperature for approximately four days.

The source of the eggs from the feces was determined by dissecting the newts.

The feces and newt were discarded if specimens other than *P. salamandra* were found. The results of sixty-eight such dissections showed that 63 per cent were infected only with *P. salamandra,* 16 per cent with only *P. parva,* and 21 per cent with both species or not infected.

One snail of each species, *P. columella* and *H. anceps* was killed and fixed six hours after it was certain that ingestion of eggs containing active miracidia had occurred. In serial sections of *P. columella,* fourteen eggs were counted in the intestine from one of which a moribund miracidium had hatched. In serial sections of *H. anceps,* five eggs were counted in the intestine. Other snails of both species which ingested eggs containing active miracidia were killed and fixed at the end of twenty-four and thirty-six hours respectively. At both times miracidia were found in the lumen of the intestines of *P. columella.* In the latter case (36 hours), miracidia were found attached to the outer intestinal wall. Eggs in varying stages of disintegration were found in the intestines of *H. anceps.* However, snails of this species were not infected.

Five adult *Pseudosuccinea columella,* collected in the field and isolated in the laboratory for thirty to forty days until it was certain that no cercariae were emerging, were fed eggs containing active miracidia. There was 100 per cent infection of the five snails. Four snails were sacrificed; one was kept under periodic observation and cercariae emerged from this mollusc in 28 days.

The snails which ingested mature, but not active, miracidia did not become infected. Thirteen of these eggs were counted in the intestine of one snail. The opercula were free from four of the eggs, but no miracidia were found.

All attempts to hatch eggs free of the body of the snail were unsuccessful.

B. Sporocysts

1. *Primary* (Plate I, Fig. 4)

Sectioned material of infected laboratory raised specimens of *P. columella* shows that the primary sporocysts are localized in the lymph spaces adjacent to the outer intestinal wall.

2. *Secondary* (Plate I, Fig. 5)

Sectioned material of infected laboratory-raised specimens shows many daughter or secondary sporocysts in the liver. Many remnants of primary sporocysts persisted in the lymph spaces adjacent to the outer intestinal wall.

A morphological study was made and is discussed under the descriptions of the stages.

C. Metacercaria

(Plate I, Fig. 7)

Four dragonfly naiads of the genera *Sympetrum* and *Leucorhinia,* one adult aquatic insect of the family NOTONECTIDAE, four *P. columella* and four *Helisoma anceps* were placed in separate stender dishes containing active cercariae. Laboratory reared snails were likewise exposed to cercariae. Cercariae were passively taken into the branchial chamber of the dragonfly naiads. Although they were constantly observed for a period of six hours, no cercariae were actually seen to penetrate any of the odonatans. When dissected after an exposure of eighteen hours, approximately one hundred metacercariae were recovered from each of the dragonfly naiads.

In one case they were easily seen through the transparent chitinous exoskeleton of a recently molted naiad. Dissection of the adult back-swimmer, after eighteen hours exposure to cercariae, yielded seven recently encysted metacercariae. Metacercariae were found encysted in the foot of *P. columella.* These were in the same stage of development as those from the insects. No metacercariae were found in *Helisoma anceps.*

Another set of the same organisms plus an adult diving beetle were exposed to cercariae for eighteen hours, after which they were removed and set aside for dissection or fixation thirty-eight hours later. Fewer metacercariae were recovered from the dragonfly, but those recovered were distributed throughout the abdominal and thoracic regions, with the greater number encysted among the gill lamellae of the branchial chamber. Fourteen were teased out of the foot of *P. columella. H. anceps* yielded negative results. Three metacercariae were obtained from the adult diving beetle. The sectioned material shows encysted metacercariae in all but *Helisoma* snails.

The freshly dissected dragonfly larvae, containing numerous encysted metacercariae was fed to a newt which had been kept in an aquarium for four months. At the end of two days the newt was sacrificed and eleven immature flukes were recovered from the upper part of the intestine (Plate I, Fig. 8). This experiment was repeated with another newt which was fed a small live dragonfly naiad that had been exposed to cercariae daily for one week; forty-two immature flukes were recovered. Eleven and one-half days after feeding, another newt was dissected and thirty-one flukes were recovered from the intestine. These flukes, though immature, were larger and more developed than those previously secured (Plate I, Fig. 9). No flukes were present in the intestine of the salamander used as a control.

IV. DESCRIPTION OF STAGES

A. Morphology of Sexually Mature Adult

(Plate I, Fig. 1)

Specimens of *Plagitura salamandra* are flattened dorso-ventrally, and are elongate oval in outline. A fixed and mounted specimen, selected as representative, measures 3.18 mm in length and 0.9 mm in width at the acetabulum. The oral sucker is slightly subterminal and measures 0.21 mm in diameter. The acetabulum is situated in the posterior end of the anterior third of the body and measures 0.26 mm in diameter. The pharynx is posteriorly adjacent to the oral sucker and is 0.09 mm in length. The oesophagus is thin-walled and extends posteriad for a distance of 0.44 mm to unite with the intestinal caeca. The intestinal caeca, which are relatively shorter than in *P. parva,* terminate just anterior to the testes. The ovary is located just posterior to the acetabulum and, in the selected specimen, is just to the right of the mid-line. In other specimens, it varies from median to left-median. The shape of the ovary also varies. In the selected specimen, it is oval with a length of 0.22 mm and width of 0.14 mm and is slightly lobed, not notched, in its contour. The vitellaria occupy the middle third of the lateral borders, the follicles are numerous and fairly large, extending slightly over the intestinal caeca. The vitellaria extend from the fork of the intestinal caeca to the posterior margin of the testes. There are two lateral yolk ducts which join to form the transverse yolk duct, which is ventral to the intestinal caeca. The transverse yolk ducts unite between the

seminal receptacle and the ovary to form the common yolk duct which in turn joins the oviduct. The coiled uterus extends caudally from the oviduct and consists of an intricately coiled tube filled with eggs. The uterus descends on the left side, at first, but crosses over to the right side before reaching the middle of the body; it is extensively coiled and proceeds to the testes. The ascending uterus is coiled and proceeds forward on the left until near the middle of the intestinal caeca. Here it crosses to the right and continues anteriorly until it reaches the region of the ovary where it crosses to the left and extends midway to the center line of the acetabulum to join with the muscular metraterm. The metraterm terminates in the common genital atrium and genital pore.

A conspicuous cirrus sac, small cirrus and seminal vesicle are located just anterior to, and overlapping, the acetabulum. Their position to the right or left of the median line varies in direct relation to the ovary. The two testes are lobed and have an average diameter of 0.21 mm by 0.29 mm. The testes are smaller in relation to the body than are those of *P. parva*. Vasa efferentia arise from the testes, run anteriorly, join dorsal to the acetabulum forming the vas deferens. The vas deferens enters the muscular cirrus sac before emptying into the seminal vesicle. The cirrus sac is pear-shaped and terminates in the genital atrium. This genital atrium connects with the exterior through a genital pore, which is median and just posterior to the forks of the intestinal caeca.

The excretory vesicle is not clearly shown in stained toto mounts. The typical Y-shaped excretory vesicle pattern of the family is clearly recognized in living flukes, especially young, immature ones.

Minute spines beset the cuticula in the anterior third of the body; they are much closer together in the region of the oral sucker.

B. Eggs and Miracidia

(Plate I, Figs. 1 and 2)

The color of uterine eggs varies in relation to their maturity, being dark brown within the metraterm and becoming lighter in color as they extend toward the ovary. The shell is thin and somewhat pliable. The ovoid, symmetrical, operculated egg averages 0.031 mm by 0.020 mm.

No definite miracidium was visible in an egg taken from the uterus. Eggs taken from recently passed feces of newts show miracidia, poorly formed and irregular in shape. As has been mentioned, these larvae are not active or infective until they are allowed to remain in pond water for four days. Because of the opacity of the shell no definite structures are clearly visible, but germ masses are seen in the posterior region. Active miracidia when forcibly hatched are fairly regular in shape, measuring 0.035 mm by 0.022 mm in size. These miracidia are colorless, and are covered with long cilia in the anterior portion. Germ masses or individual cells lie haphazardly scattered in the posterior end and are clearly differentiated by staining with Janus Green.

C. Sporocysts

(Plate I, Figs. 4 and 5)

Sporocysts were found in the lymph spaces adjacent to the intestine and in the liver of heavily infected *P. columella*. The primary sporocysts removed from the

intestinal region are formed from the miracidia and are similar to the type described by Stunkard for *P. parva*. The mother sporocyst is irregularly sac-like in shape, with a thin membranous wall inclosing numerous germ masses. There is little in the way of a definite shape or recognizable structures in either living sporocysts, stained toto mounts, or sectioned material. The germ masses mature with a resulting increase in the size and an irregularity in shape of the mother sporocyst. The size of these sporocysts ranges from 0.03–0.12 mm in length by an average of 0.03 mm in width. The sporocyst from *P. columella* shown in Plate I, Fig. 4, is a full-grown larval form. The daughter sporocysts escape through a birth pore, which is located at one end of the sporocyst, and migrate to the liver.

The sporocysts from the liver of *P. columella* are secondary or daughter sporocysts (Plate I, Fig. 5). These sporocysts are generally larger, thin-walled, and oval to vermiform in shape. They are very numerous and are embedded in the interlobular connective tissue of the digestive gland. They range from 0.24–1.0 mm in length by 0.03–0.35 mm in width. Within the transparent walls are clusters of germ masses that become differentiated and clustered into cercaria-like larvae toward the anterior end. The germ masses in the posterior region are similar in appearance to those within the primary sporocysts. A definite birth pore is located in the anterior end, and cercariae were seen to emerge through it.

D. Cercariae

(Plate I, Figs. 6 and 6a)

The cercariae undergo rather rapid development from the time that they leave the daughter sporocyst until they finally escape from the shell of the snail. Cercariae were observed emerging from the small pores surrounding the base of the apex-spiral of the shell of *P. columella*. When the temperature of the water was raised to 22.2° C, large numbers of the cercariae literally fought their way out of the pores. When the temperature was lowered to 11.1° C, no cercariae were seen to emerge for a period of thirty-five minutes.

The behavior of the cercariae of *P. salamandra* is similar to that described for those of *P. parva*, by Stunkard (1936).

The body of the cercariae is an elongate oval, slightly narrowed anteriorly, but varies considerably in the living specimens because of the constant muscular contractions. The measurements of twenty-five living specimens showed a range of 0.23–0.32 mm by 0.102–0.185 mm. The oral sucker and the acetabulum are approximately the same diameter; 0.054 mm is the average diameter of twenty-five specimens measured, although, in a few specimens, the acetabulum is considerably smaller than the oral sucker. The acetabulum in all cases, is situated just behind the middle of the body. In the dorsal portion of the oral sucker is a conspicuous stylet which measures 0.016 mm in length and 0.0054 mm in width (Plate I, Fig. 6a). When the body of the cercaria is contracted, the stylet, with the point up, is pulled down into the cuticula, but when the organism extends its body forward, the last motion it makes before full extension is a quick jerk, or thrust, which extends the stylet beyond the surface of the body. The straight, cylindrical shaft is hollow and terminates in a sharp point.

Living specimens stained with Janus Green or neutral red show structures more clearly than do fixed and stained preparations, although the latter were used to check

measurements. Eight penetration glands, with an average diameter of 0.018 mm, could be distinguished in the mass of cells surrounding the acetabulum. The ducts from these glands run anteriorly. The cystogenous glands lie under the cuticula of the posterior two-thirds of the body.

The mouth is slightly subterminal and opens into a bulbous pharynx, which is surrounded by a mass of cells that stains deeper than the adjoining mass of cells. A narrow oesophagus, 0.0048 mm in width, extends posteriorly to bifurcate just anterior to the acetabulum. The three posterior excretory vesicles are arranged triangularly and are joined by narrow canals to form the characteristic Y-shaped bladder. Their position is continually altered by the contractile nature of the walls so that they are seldom seen simultaneously. The fluid material which accumulates in the posterior bladder is eliminated through the posterior excretory pore located at the base of the tail.

The tail of the cercaria of *P. salamandra* is attached subterminally to the body and measures 0.15–0.306 mm by 0.041–0.045 mm. A slight dorso-ventral fin, which flares out slightly at the distal end, runs the entire length of the tail (Fig. 6).

E. Metacercariae

(Plate I, Fig. 7)

Metacercariae are spherical to ellipsoidal in shape. The cyst wall which is laid down by the secretions of the peripheral cystogenous glands, is thin and colorless. The average measurements of twenty-five cysts were 0.14 mm by 0.10 mm. In these metacercariae, cercarial structures are recognizable. Six metacercariae were seen with the tail included within the cyst wall. When examined twelve hours later, the tail had disappeared. After twelve hours the stylet is still attached to the dorsal lip of the oral sucker.

The older metacercariae do not increase in size. The posterior excretory vesicle is the most evident structure; it appears as a dark Y-shaped vesicle filled with granular concretions. The stylet is detached and in some specimens is seen lying in the cyst. There is a very slight increase in the thickness of the cyst wall. Forcibly excysted metacercariae, at this stage, are similar in structure to the naturally excysted metacercariae removed from the intestine of the newt.

V. DISCUSSION

Holl (1928) erected the genus *Plagitura* and placed in it a new species, *P. salamandra,* which he described from the intestine of *Triturus viridescens.* He stated that his original specimens measured 1.5–4.0 mm in length, and lacked cuticular spines. Kelley (1934) and Stunkard (1936) have both examined co-types of Holl's specimens and are in agreement that spines are present. In the living specimens examined in the course of this investigation all have exhibited regularly alternating rows of spines.

Stunkard (1933a) listed the specific characters of *P. salamandra* and *P. parva;* he compared the morphological differences between the two species.

This study indicates slight differences in the morphology of the larval forms. The cercarial stylet of *P. parva* is provided with dorso-ventral and lateral protuberances at the base of the anterior cone; the stylet of *P. salamandra* is without such protuberances. The tail of the cercaria of *P. parva,* according to Stunkard's description

is without the slight dorso-ventral fin-fold, whereas such a fold is present in *P. salamandra.*

The data afforded by life cycle studies are of the utmost importance in taxonomic considerations. In the life history of *Plagitura salamandra* the eggs must first be ingested by *Pseudosuccinea columella.* In the life cycle of *Plagitura parva* they must be ingested by *Helisoma anceps.* Stunkard was unsuccessful in attempts to infect *Helisoma* with eggs from *Plagitura salamandra.* This result was confirmed in this study. The cercariae of *P. parva* and *P. salamandra* both utilized the snail *P. columella* and insect larvae as second intermediate hosts. Metacercariae of *P. parva* have been reported from dobson-fly larvae, while metacercariae of *P. salamandra* occur in dragon-fly larvae and in adult aquatic insects. Both species of adult trematodes are found in the small intestine of *Triturus viridescens.*

Definite conclusions can be drawn at this time as to the bionomic distinction between the two species. Stunkard (1936) presented data to support the specific identity of *P. parva* as it is compared with *P. salamandra.* This investigation presents data to support the specific identity of *P. salamandra* as it is compared with *P. parva,* and thus indicates the validity of Stunkard's conclusions as to the separation of the two species.

VI. SUMMARY

The life history of *Plagitura salamandra* Holl, 1928 is traced and all stages in the cycle are described. The life cycle of *Plagitura salamandra* Holl, 1928 is compared with the life cycle of *Plagitura parva* Stunkard, 1933. Data are presented to support the specific identity of the two species.

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EXPLANATION OF PLATE I, P. 562

bp	birth pore	gm	germ masses
ce	cercaria	os	oral sucker
cg	cystogenous glands	pg	proteolytic glands
ev	excretory vesicle	s	stylet

Figures 1 through 9 = *Plagitura salamandra*. All drawings except Fig. 8 were made with aid of a camera lucida.

FIG. 1. Dorsal view of a representative specimen.

FIG. 2. Fully developed egg.

FIG. 3. Forcibly hatched miracidium to show manner of emergence.

FIG. 4. Outline drawing of a mature primary sporocyst.

FIG. 5. Outline drawing of a mature secondary sporocyst.

FIG. 6. Cercaria, ventral view, outline from stained specimen, details from many living larvae.

FIG. 6a. Stylet, basal part of shaft not shown.

FIG. 7. Metacercaria seven days after encystment in dragonfly naiad.

FIG. 8. Metacercaria, a composite drawing of a forcibly excysted metacercaria and a naturally excysted metacercaria taken from the intestine of the newt.

FIG. 9. Specimen recovered from the intestine of newt eleven and one-half days after metacercaria had been ingested.

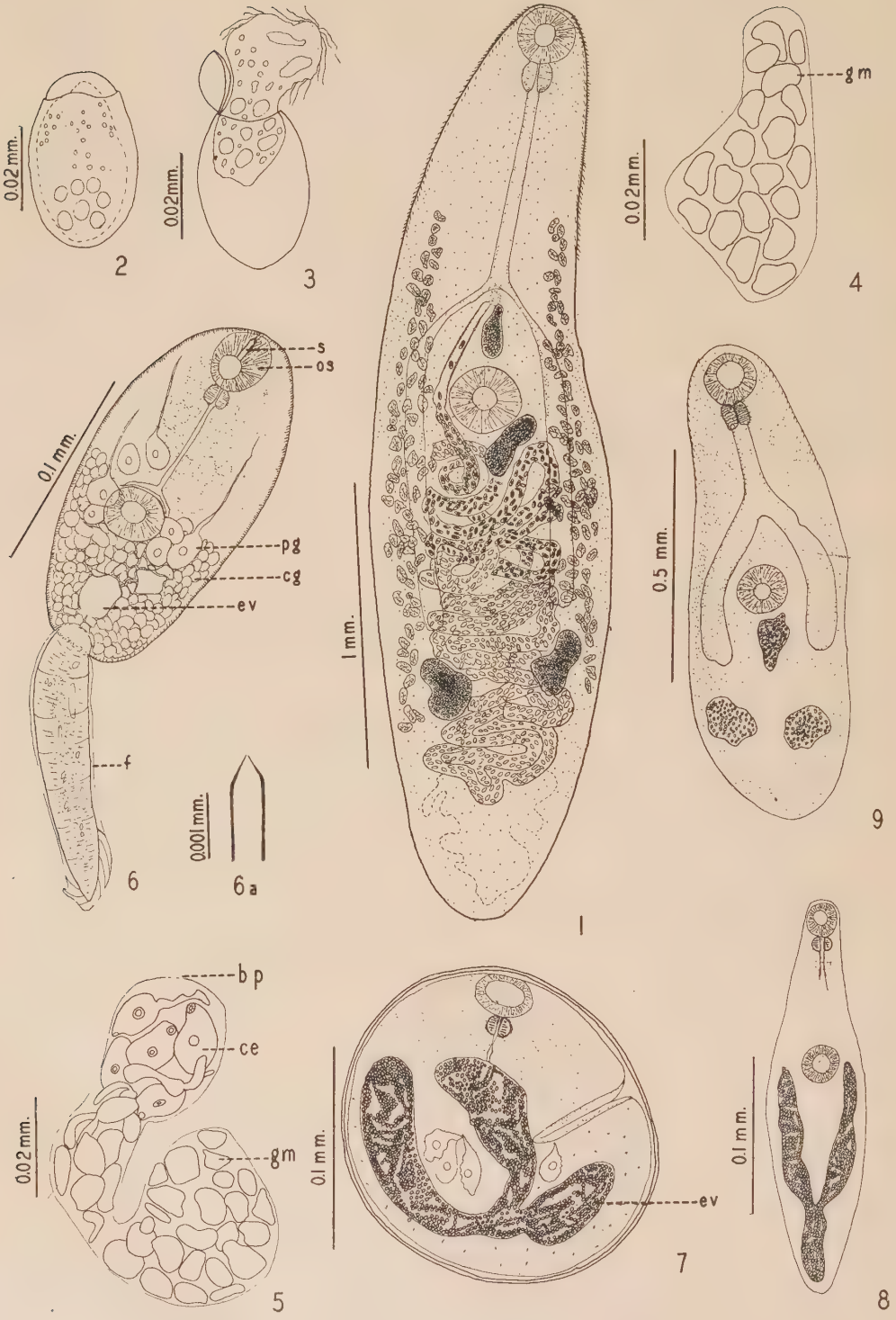
0.02 mm.
2
0.02mm.
3
1 mm.
1
0.02mm.
gm
4
s
os
0.1 mm.
pg
cg
ev
f
6
0.001 mm.
6a
0.5 mm.
9
bp
ce
0.02 mm.
gm
5
0.1 mm.
ev
7
0.1 mm.
8

PLATE I

MICROAGGLUTINATION TESTS WITH *TRICHOMONAS VAGINALIS*

RAY E. TRUSSELL*

INTRODUCTION

The immunology of human vaginal trichomoniasis has received scant attention. Evidence of circulating antibodies against *Trichomonas vaginalis* has been provided by two studies. Using an alcoholic extract of an impure culture of the protozoa as an antigen, Wendlberger (1936) demonstrated complement-fixing reactions with the sera from twenty-two of thirty-two infected women. Sixteen uninfected women gave negative reactions, as did carriers with no manifestations of vaginal disease. The twenty-two women whose sera gave positive reactions had abnormal vaginal discharges with or without vaginitis.

Trussell and Wilson (1942) prepared an antigen consisting of a saline suspension of bacteria-free *T. vaginalis* that had been cultured in a simple peptone-carbohydrate human serum medium, and repeatedly washed before use, thus insuring a preparation in which the only complex elements foreign to the human body were the components of the trichomonads. Ante partum venous blood specimens from four hundred women, who were examined for trichomoniasis, were tested for complement-fixing antibodies. The results are tabulated as follows:

Complement-fixation reactions in four hundred ante partum obstetric patients

Patients	No.	Positive complement fixation
With *T. vaginalis*	110	52 (47.27 per cent)
Without *T. vaginalis*	290	48 (16.55 per cent)

Patients infected with *T. vaginalis* were three times as likely to have positive serologic reactions as were women who could not be shown to be infected at the time of the test. Further evaluation of the data showed that women with clinical evidence of the infection were more likely to give positive serologic reactions. However, there was a considerable percentage of reactors among patients with no objective pathologic changes in the vagina, which might be due to previous infection. Cross-reactions with oral and intestinal trichomonads were not excluded. No practical importance is attached to the demonstration of complement-fixing reactions at this time.

It is the purpose of the present report to discuss microagglutination tests demonstrable in sera from experimentally immunized rabbits and in human blood specimens drawn from syphilitic or pregnant women.

EXPERIMENTAL METHODS

Immunization Procedures

1. *Preparation of antigen.*—The original bacteria-free strain of *Trichomonas vaginalis* (Trussell 1940) was cultured in C.P.L.M. medium made as described in

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the appendix but modified from previous descriptions (Johnson and Trussell, 1943; Trussell and Johnson, 1945) by the substitution of rabbit serum for human serum and fresh rabbit liver infusion made according to Huddleson's (1943) directions instead of the commercial liver preparation. Therefore, organic components of the medium used for production of the antigen were cysteine, maltose, rabbit serum, rabbit liver infusion, peptone and agar. After the protozoa had been cultured in this medium for 2 days at 35° Centigrade they were washed three times with Ringer's solution by centrifugation and resuspension, counted in a hemocytometer chamber, and diluted to the desired number of flagellates per cubic centimeter.

This suspension of living protozoa was used either as such, or after the addition of final concentrations of 1 : 2,000 formalin and 1 : 20,000 phenyl mercuric borate.

2. *Routes of administration of antigen, dosages, and schedule followed.*—Reference to Table 1 will show that 5 rabbits received living protozoa intravenously, 3 received killed flagellates intravenously, and 2 were given killed organisms subcutaneously. The time elapsed during the period of immunization was 13 weeks. Either 5 or 6 blood specimens drawn by cardiac puncture were collected from each rabbit. The approximate number of protozoa injected into each animal ranged from 42.5 million to 74.5 million.

Testing Method

1. *Preparation of agglutinating antigen.*—When *Trichomonas vaginalis* is cultured in C.P.L.M. medium containing small amounts of dispersed agar the protozoa grow in clumps. Such clumps are difficult to disrupt and when incorporated in an agglutinating antigen, serve as a source of error. To eliminate this natural clumping the flagellates were grown in C.P.L.M. medium in which 0.5% gelatin was substituted for agar. In addition, peptone was omitted because of the theoretical possibility that it as well as the agar might have entered into some antigenic complex which would give rise to confusing reactions during the subsequent testing of sera from the immunized rabbits.

It was found that antigens prepared in medium containing human serum and commercial liver infusion gave the same microagglutination titers as those prepared in medium made with rabbit serum and rabbit liver infusion. For the sake of convenience human serum and commercial liver infusion were used to prepare antigens used for the microagglutination tests to be tabulated below. This might not be advisable if such technics as the complement fixation test or precipitin test were to be investigated.

While large inocula (0.5 to 1.0 cc) were required to obtain satisfactory growth after 2–3 days in this modified medium, the flagellates were well dispersed, and after having been washed 3 times with M/15 Na_2HPO_4, filtered through cotton, counted and suspended in the same diluent to a final count of approximately two million organisms per cubic centimeter, they presented a "smooth" suspension which was suitable for microagglutination tests. The antigen was preserved with 0.3 per cent formalin.

2. *Macroagglutination tests.*—Prior to use, all sera described in this report were heated at 56° Centigrade for 20 minutes. Using M/15 Na_2HPO_4 as the diluent of both sera and antigens, macroagglutination tests were studied first by the usual technics. It was found that in both sera from experimentally immunized animals and from humans, clumping did occur and that the demonstrable titers could be

TABLE 1.—*Summary of dosages, time intervals between injections and microagglutination titers obtained in serial blood specimens from ten rabbits immunized against* Trichomonas vaginalis. *Rabbits 1–5 received living organisms intravenously, rabbits 6, 7, and 8 received dead protozoa intravenously, and rabbits 9 and 10 were given killed flagellates subcutaneously*

Rabbit 1				Rabbit 2				Rabbit 3				Rabbit 4				Rabbit 5			
Cumulative dosage in millions	Day injected	Day bled	Titer	Cumulative dosage in millions	Day injected	Day bled	Titer	Cumulative dosage in millions	Day injected	Day bled	Titer	Cumulative dosage in millions	Day injected	Day bled	Titer	Cumulative dosage in millions	Day injected	Day bled	Titer
0.0	0	0	1 : 20	0.0	0	0	1 : 80	0.0	0	0	1 : 40	0.0	0	0	1 : 40	0.0	0	0	1 : 40
2.5	7	14	1 : 160	2.5	7	14	1 : 160	2.5	7	14	1 : 160	2.5	7	14	1 : 160	2.5	7	14	1 : 320
6.5	14			6.5	14			6.5	14			6.5	14			6.5	14		
10.5	21	25	1 : 320	10.5	21	25	1 : 2560	10.5	21	25	1 : 1280	10.5	21	25	1 : 1280	10.5	21	25	1 : 320
14.5	26			14.5	26			14.5	26			14.5	26			14.5	26		
18.5	33	37	1 : 320	18.5	33	37	1 : 2560	18.5	33	38	1 : 2560	18.5	33	38	1 : 2560	18.5	33	38	1 : 5120
24.5	40			24.5	40			24.5	40			24.5	40			24.5	40		
32.5	47			32.5	47			32.5	47			32.5	47			32.5	47		
42.5	54	59	1 : 1280	42.5	54	59	1 : 5120	42.5	54	60	1 : 5120	42.5	54	60	1 : 5120	42.5	54	60	1 : 10,240
74.5	82	87	1 : 640	74.5	82	87	1 : 2560	74.5	82	90	1 : 2560	74.5	82	90	1 : 5120	74.5	82	90	1 : 1280

Rabbit 6				Rabbit 7				Rabbit 8				Rabbit 9				Rabbit 10			
0.0	0	0	1 : 20	0.0	0	0	1 : 40	0.0	0	0	1 : 40	0.0	0	0	1 : 40	0.0	0	0	1 : 40
2.5	7	14	1 : 160	2.5	7	14	1 : 160	2.5	7	14	1 : 80	2.5	7	14	1 : 160	2.5	7	14	1 : 40
6.5	14			6.5	14			6.5	14			6.5	14			6.5	14		
10.5	21	25	1 : 320	10.5	21	25	1 : 1280	10.5	21	25	1 : 320	10.5	21	25	1 : 320	10.5	21	25	1 : 80
14.5	26			14.5	26			14.5	26			14.5	26			14.5	26		
18.5	33	38	1 : 320	18.5	33	39	1 : 640	18.5	33	39	1 : 640	18.5	33	39	1 : 80	18.5	33	39	1 : 80
24.5	40			24.5	40			24.5	40			24.5	40			24.5	40		
32.5	47			32.5	47			32.5	47			32.5	47			32.5	47		
42.5	54	60 Died	1 : 320	42.5	54	65	1 : 640	42.5	54	65	1 : 320	42.5	54	66	1 : 80	42.5	54	66	1 : 80
				62.5	82	90	1 : 640	62.5	82	90	1 : 320								

fairly well duplicated in repeated tests. From a practical point of view, however, the clumps of protozoa were very unstable and the endpoints of the titrations were difficult to read. As a result the technic was discarded in favor of a microagglutination test.

3. *Microagglutination tests.*—The antigen antibody mixtures were prepared in M/15 Na_2HPO_4 in exactly the same manner as for the macroagglutination tests by mixing 0.5 cc of the various dilutions of each serum to be tested with 0.5 cc of antigen (2 million protozoa per cc). Immediately after thorough shaking, 0.02 cc of each mixture was pipetted into a wax-pencilled square on a glass slide. As many as 12 tests were made on each slide. The slides were placed on supports in Petri dishes containing moistened filter paper in the bottom and incubated 4 hours at approximately 37° Centigrade. Each drop was then examined under the low power lens of the microscope.

A negative test was considered to be one in which there was no evidence of clumping (See Figure a, Plate I). Positive tests were those in which varying degrees of clumping of the protozoa occurred (See Figures b and c, Plate I). The endpoint was given as the last dilution of the serum in which a few small clumps occurred. The observed titers were usually reproducible in repeated tests and never varied more than one tube dilution.

Microagglutination Titers Demonstrable in the Sera of Experimentally Immunized Rabbits

Table 1 illustrates the rising microagglutination titers which developed in the sera of the experimental animals. It also suggests that living organisms injected intravenously produced higher agglutinin titers than did killed flagellates given intravenously. However, the latter procedure was more efficacious than subcutaneous inoculation of the dead protozoa.

Microagglutination Titers Demonstrable in Human Sera

The 200 human blood sera which were tested were evenly divided between those from pregnant women and from syphilitic women. These specimens were chosen because such women have a high incidence of vaginal trichomoniasis and this was a preliminary survey designed to examine the technical suitability of the test and to demonstrate agglutinating titers, if any, in human sera. The individual histories of the patients are not known. Table 2 summarizes the results of the tests.

TABLE 2.—*Microagglutination titers against* Trichomonas vaginalis *demonstrable in the sera of pregnant and female syphilitic patients*

Titer	Pregnant patients	Female syphilitic patients
Negative	90	92
1 : 10	8	5
1 : 20	2	2
1 : 160		1
Total	100	100

Cross Reactions between Bacteria-free Strains of Trichomonas vaginalis

The experiments described above all utilized antigens prepared from the original bacteria-free strain of *T. vaginalis* isolated in 1939. In 1945 seven additional bacteria-free cultures of the protozoon were developed with the aid of penicillin by

Johnson, M. Trussell and Jahn (1945). Common antigens which might be demonstrable between these strains of the flagellates would be of taxonomic interest and would also enter into any consideration of vaccines.

Strains 2 and 4 of the seven cultures mentioned were available and were tested with antisera developed against the original (1940) strain. Close antigenic relationships were demonstrated between the 3 cultures. For example, serum number 4 (drawn at 60 days) agglutinated all three strains at titers of 1 : 2560–1 : 5120 and serum number 5 (drawn at 60 days) agglutinated all three at a titer of 1 : 10,240.

Agglutinin absorption tests revealed the same relationship. Absorption of agglutinins from 1.0-cc portions of serum 5 by exposure to three successive doses of 4 million organisms each of strains 2 and 4 for two hours at 37° Centigrade reduced the agglutinating titer of that serum to 1 : 20 and 1 : 160 respectively when the original stimulating antigen was used as the test organism. Similarly single doses each of 18 million protozoa representing strains 2 and 4 when added to 1.0 cc of serum 4 and incubated 2 hours at 37° Centigrade and overnight in the refrigerator reduced the titer from 1 : 10,240 to 1 : 320 and 1 : 640 respectively.

SUMMARY

The experiments described indicate that *Trichomonas vaginalis* can be studied by known serologic technics, that the protozoon stimulates the production of antibodies in experimentally inoculated animals, that similar antibodies exist in low titer in the sera of a small percentage of pregnant or syphilitic women, and that common antigens exist in the 3 available bacteria-free strains of the flagellate which were tested. The latter observation has therapeutic and taxonomic implications.

ACKNOWLEDGMENT

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APPENDIX

C.P.L.M. medium (cysteine-peptone-liver-maltose)

Bacto peptone	32.0 gm
Bacto agar	1.6 gm
Cysteine HCl	2.4 gm
Maltose	1.6 gm
Liver infusion (Difco, make as per manufacturer's instructions)	320.0 cc
Ringer's solution (NaCl—0.6%, $NaHCO_3$, KCl and $CaCl_2$—0.01% each)	960.0 cc
Sodium hydroxide N/1	11–13 cc

Heat mixture in a boiling water bath to melt the agar; filter through coarse paper; add 0.7 cc of 0.5 per cent aq. methylene blue; adjust pH to 5.8–6.0 with N/1 HCl or N/1 NaOH; tube in 8-cc amounts in medium-sized tubes; plug with cotton and autoclave. After cooling, add aseptically 1 or 2 cc of sterile (filtered) human serum. Incubate at least 4 days. This medium is stored at room temperature and is good usually for two to three weeks, or as long as an amber "anaerobic" zone is evident. Cork stoppers are advantageous.

Growth in this medium is luxuriant, especially in the presence of maltose. A population of two to four million organisms per cubic centimeter is a common finding after two days' incubation at 35–37° Centigrade. However, subcultures of about 0.05 cc must be made every other day; otherwise so much acid will be produced that the protozoa cannot survive.

Plate I

Figure a. Negative microagglutination reading with *Trichomonas vaginalis*.

Figure b. Strongly positive reaction.

Figure c. Smaller clumps seen in higher dilutions of serum.

PLATE I

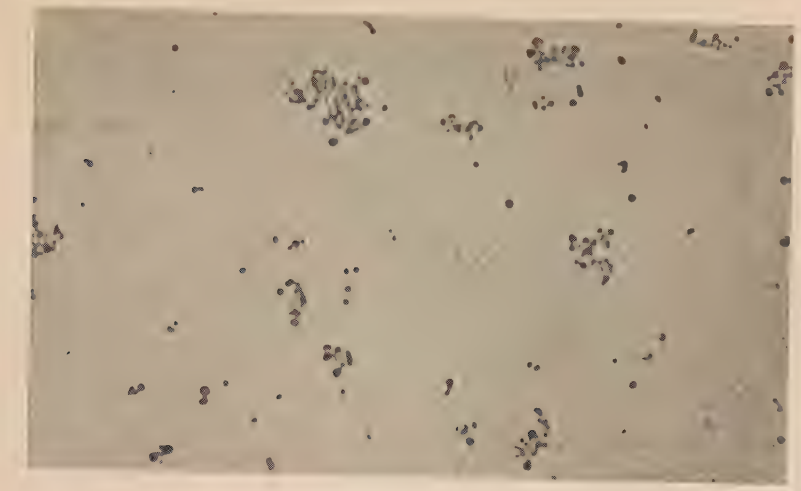

Figure c

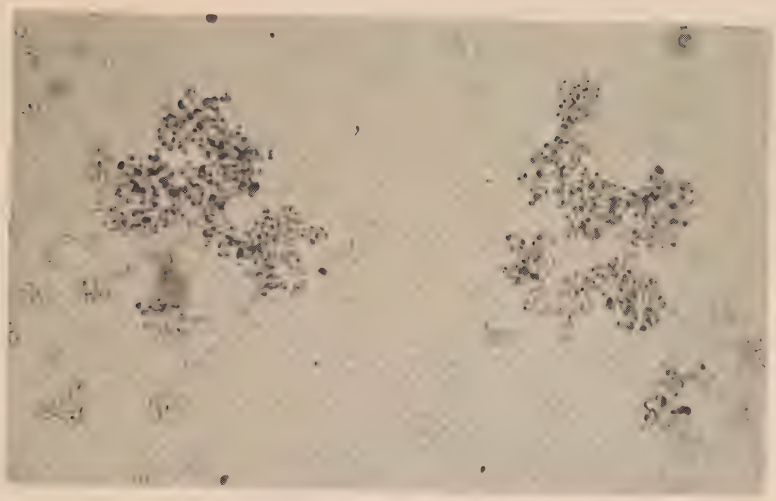

Figure b

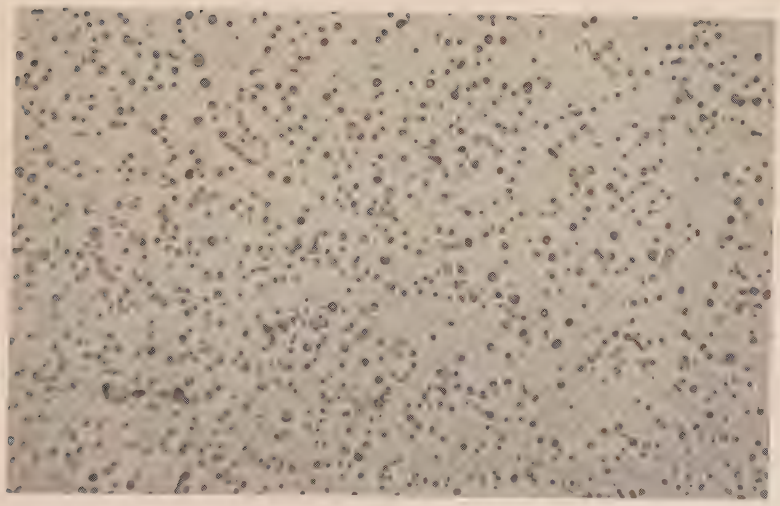

Figure a

OBSERVATIONS ON THE RATE OF GROWTH AND MATURITY OF *LITOMOSOIDES CARINII,* A FILARIAL WORM OF THE COTTON RAT[1]

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In the course of experiments leading to the production of infections with the filarial worm, *Litomosoides carinii,* in the cotton rat, *Sigmodon hispidus texianus,* a few observations have been made on the size of the worms at various ages. That these worms reach sexual maturity within 50 days and appear to have completed their growth in $2\frac{1}{2}$ months is somewhat surprising since epidemiological evidence has indicated a very long period of growth for the filariae infecting humans and domestic animals.

METHODS AND MATERIALS

In the first experiments, freshly gathered grass nests of cotton rats were placed on a layer of soil in a sheet iron box along with an infected cotton rat and her newborn young. The details of these early experiments were reported (Scott and Cross, in press) at the meeting of the American Society of Tropical Medicine in November 1945. It was at this same meeting that the vector of this parasite became known (Williams and Brown, 1945). Since that time our methods have differed slightly to take advantage of this new information. A colony of tropical rat mites, *Liponyssus bacoti,* was placed in each box with an infected rat early in her gestation period, so that the mites would become infected by the time the young rats were born. In addition three grown rats were used in the experiments. They had been born in the laboratory and raised in a mite-free room where autopsy of controls showed that no infection took place. They were put into boxes containing mite colonies which had fed on infected cotton rats for several weeks. No correlation can yet be detected between the rate of growth of the worms and the age of the rats.

After the rats had been in the boxes for 10 days or longer, they were removed, freed of mites, and held in mite-free cages for autopsy at various intervals. The worms recovered were fixed in 70 per cent alcohol held at approximately 70° C. The small worms were then mounted in glychrogel (Zwemer, 1933), and were measured under the microscope with a calibrated ocular micrometer. The large worms were kept in alcohol or alcohol-glycerin mixture. Their images were projected on a paper screen along with a scale for calibration. Tracings of these images were measured with a map measuring device.

These methods are obviously somewhat deficient in that it is not possible to determine the exact age of the worms. The maximum age is definitely established, however, by the time from the first exposure to the date of autopsy. For this reason only the size of the largest worm of each sex from each animal has been recorded. If infection occurred on the first day of exposure, the size of this largest worm would tend to be greater than the average for that age. If infection did not occur until

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later, however, the recorded size would tend to be smaller than the true average for the age indicated.

RESULTS

The results obtained by measuring the largest worm of each sex from each rat are plotted in Fig. 1. The curves are logistic growth curves fitted by inspection to represent what seems to be the most likely average growth rate on the basis of such information as is now available. In drawing these curves consideration was given to two types of information presented in a previous report (Cross and Scott, in press). In the first place, the worms of both sexes were found to average about 7 mm in length when they moulted into the adult stage. Consequently the curves were

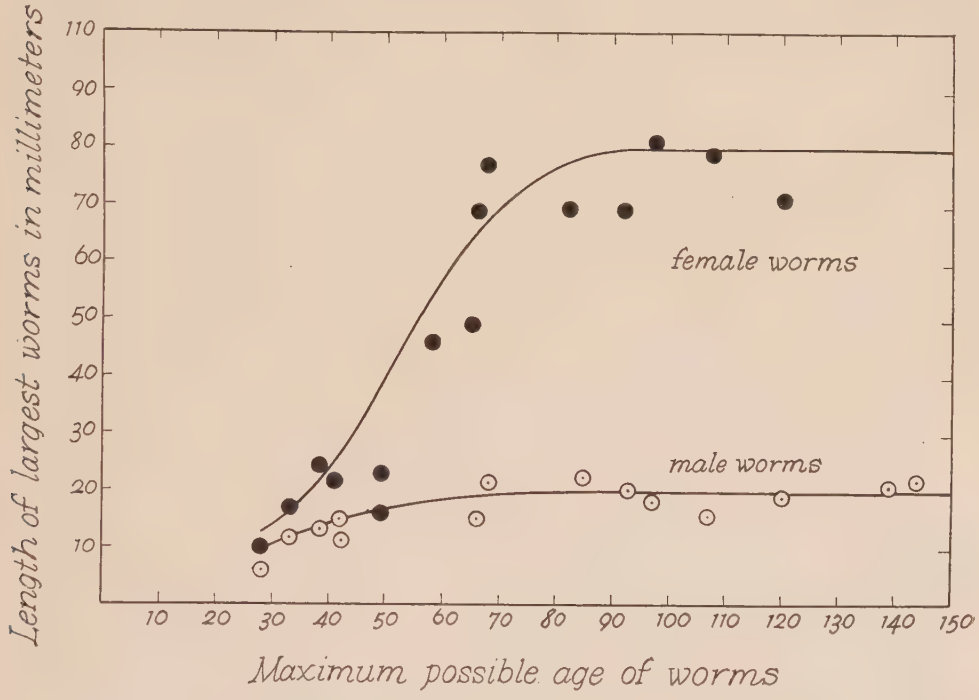

FIG. 1. Showing the length of the largest worm of each sex from each animal in relation to the maximum possible age, i.e., the time from the first exposure to autopsy. The curves represent the best available estimate of the average growth rates for the two sexes.

drawn so as to indicate that this cycle of growth had started at 7 mm. In the second place, the same report compares the size of the mature worms in our collections with those reported by other authors. We have collected several thousand worms from naturally infected rats caught in the fields of the mainland portion of Galveston County, Texas, have measured representative samples of the worms, and have also selected from many infections the largest worms of each sex for measurement. In brief, we have found that the largest female worms are usually 80 to 90 mm in length with a few specimens as long as 100 mm. With two exceptions these figures are similar to those reported elsewhere. Chandler (1931) found that the female worms, taken from this same host species captured within 30 or 40 miles of our collecting areas, measured 50 to 65 mm, while Vaz (1934) found one female from another host in Brazil to measure 120 mm. On the other hand, the largest male

worms in our collections usually measured from 19 to 22 mm with one specimen reaching 23 mm, while Chandler reported males to vary from 24 to 28 mm. Vaz and other authors have also found some males as long as 28 mm. We have observed that in wild caught rats which had been kept for a period of 6 to 12 months in the laboratory under conditions where reinfection is impossible the length of the female worms varied from 70 to 90 mm, and that of the male worms from 19 to 23 mm. On the basis of these observations and conclusions inherent in the data of Fig. 1, it has been assumed that the ultimate average size of these worms would be approximately 80 mm for the females and 20 mm for the males, and the curves were drawn accordingly. Further observations may make it necessary to change these assumptions and may show either that various strains differ with respect to the final adult size, or that the same strain may vary in this respect under different conditions.

Having settled upon upper and lower limits for the curves, it was next necessary to decide upon the best slope. In doing this, two opposing facts were kept in mind. On one hand, the points which are plotted represent the size of the largest worms and may therefore tend to be larger than the average size for the worms of the same age. On the other hand, the exact age of the worms is not known and they may often be younger, but never older, than indicated. Only when infection took place on the day the rats were placed in the boxes would the worms actually be as old as is indicated on the graph. It was therefore assumed that those cases which fell considerably below the trend of observations were probably younger than indicated, and that the very largest worms shown for any given age are probably above the average size. The lines were therefore drawn between these two extremes in an attempt to fairly balance one consideration against the other.

One further observation to be reported is that microfilariae have appeared in the blood of some of these rats as early as the 50th day. No examinations of the blood of these particular rats had been done during the week previous to this day, and it is possible that this is not the earliest date. On the other hand, other rats in the same series were examined on the 49th day and no microfilariae found.

The two rats which showed microfilariae on the 50th day were killed on the 58th and 65th days, and at that time the largest females were 46 and 49 mm long respectively. On the 50th day the fitted curve indicates the estimated size of the average female to be 40 mm. In other words it is clear that the female worms can produce viable young when they have reached no more than one-half of their maximum length. On this day the males had reached 85 per cent of their maximum length, but there is no evidence as to how long previously they had been sexually mature. Without reading from the curve beyond the observed points, it can be concluded that within a period of about $2\frac{1}{2}$ months some worms of both sexes will have reached the final size attained by the average worms.

DISCUSSION

The older literature has led us to believe that the microfilariae of *Wuchereria bancrofti* do not appear in human blood in less than one year after infection. Experience with military personnel newly arrived in endemic areas has shown that symptoms of infection can appear as early as 3 or 4 months after infection (King, 1944; Englehorn and Wellman, 1945), but up to date no reports have been seen which would warrant the assumption that microfilariae appear in such cases in less

than one year. None of the reports on experimental infections with the filariae of lower animals have included clear observations on this point. The experiments of Grassi and Noe (1900) in which dogs appear to have been experimentally infected with *Dirofilaria immitis,* have been criticized on the ground that the dogs might have been subject to natural infections. In any case their results do not give clear evidence on the point in question. Hinman (1935) attempted to infect dogs from a non-endemic area but was unable to do so. Highby (1943) found microfilariae of *Dirofilaria scapiceps* of the snowshoe hare in two domestic rabbits which had been subjected to bites of mosquitoes 286 and 391 days previously. Our finding of relatively rapid growth in *Litomosoides carinii* and sexual maturity within 50 days may be correlated with what we can assume to be the relatively short life span of the cotton rat. On the other hand, future observations may show that other species do grow more rapidly than has been supposed.

SUMMARY

Uninfected cotton rats, *Sigmodon hispidus texianus,* were exposed for a period of 10 days to colonies of tropical rat mites, *Liponyssus bacoti,* some of which were infected with the filarial worm, *Litomosoides carinii.* Upon autopsy of the rats at various intervals thereafter the largest worms of each sex were measured. On the basis of these and other observations growth curves for this species have been plotted. It is tentatively concluded that the final length attained averages about 20 mm for males and 80 mm for females. The maximum size is reached in some cases as early as 2½ months after infection. Microfilariae have been found in the blood as early as the 50th day, at which time the females have attained only one-half their ultimate size, while the males at this time have completed 85 per cent of their growth.

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EXPERIMENTS ON THE RELATION BETWEEN SEX HORMONES AND THE GROWTH OF TAPEWORMS (*HYMENOLEPIS DIMINUTA*) IN RATS

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The fact that a diet deficient in the vitamin G complex causes a stunting in the growth of the tapeworm, *Hymenolepis diminuta,* in female rats but not in male rats, reported by Chandler (1943), suggests some intricate involvement of the sex hormones with the availability or utilization of vitamins of the G complex. The inability of these tapeworms to grow in a normal manner in female rats lacking some factor or factors associated with the G complex was confirmed by Addis and Chandler (1944) and it was further shown by these writers (1946) that the nutritional factor needed by the tapeworms when growing in female rats was not any of the known components of the G complex, but was apparently present in a hydrolyzed yeast extract prepared in this laboratory. It was also shown that oral administration of liver extract reduced the stunting effect of a G-deficient diet by about one-half, indicating either that the necessary factor or factors was present in less than adequate amount, or that more than one factor was involved, not all of which existed in the liver extract. Addis and Chandler (1944) also demonstrated that a lack of the fat-soluble vitamins (A, D, and E) was responsible for a marked reduction in the percentage of worms that succeed in establishing themselves in female rats, which Chandler (1943) had noted when all vitamins were withheld.

To obtain further information on the relationship of the sex hormones to normal growth of tapeworms a series of experiments was performed on tapeworm growth in normal and castrated male and female rats, on complete and deficient diets, with and without administration of sex hormones, and also in pregnant rats and in immature males and females, with and without administration of sex hormones.

The adult rats used were, as in previous experiments, young animals 3 to 4 months old at the beginning of the experiments. Animals that were castrated or spayed were allowed a period of about four weeks for complete recovery from the operation before being placed on their experimental diets prior to infection. Infection was accomplished by oral administration of 10 artificially reared cysticercoids per rat as described in an earlier paper (Addis and Chandler, 1944). The complete diet and vitamin-deficient diet were the same as described in that paper. Autopsies were performed 14 days after infection. The results of all the experiments are shown in Table 1.

Experiment 1

Normal and Castrated Male Rats

Four groups of 6 male rats each were selected. Those of groups 1 and 2 were castrated, those in groups 3 and 4 left in normal condition. Four weeks later, when the castrated rats had entirely recovered from their operation, groups 1 and 3 were

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TABLE 1.—*Effect of sex hormones and vitamin-deficient diet on the growth of* Hymenolepis diminuta

Exp. No.	Group No.	Sex	Condition	Hormones given	Diet	Days on diet before inf.	Change in wt. of rats %	Av. No. of worms at autopsy	Length of worms (mm)				Av. width of worms (mm)	Effect on growth of worms
									Range of av. in indiv. rats	Av. per worm	Prob. errors of av.	Total per rat		
1	1	♂	Cast.	.....	C	17	+ 9.8	6.0	147–240	188	± 11.9	1127	1.07	St.
	2	♂	Cast.	.....	D	17	− 16.3	5.8	130–160	151	± 3.7	875	0.98	St.
	3	♂	Norm.	.....	C	17	+ 4.7	7.5	305–335	318	± 2.6	2390	1.58	Norm.
	4	♂	Norm.	.....	D	17	23.9	7.3	281–337	307	± 5.3	2262	1.61	Norm.
2	5	♂	Cast.	Test.p.	C	17	+ 19.4	9.8	308–385	348	± 8.0	3406	1.80	Norm.
	6	♂	Cast.	Test.p.	D	17	− 9.4	7.5	300–376	326	± 7.9	2446	1.67	Norm.
	7	♂	Cast.	M.test.	C	17	+ 28.3	8.7	261–328	300	± 6.7	2612	1.68	Norm.
	8	♂	Cast.	M.test.	D	17	0.3	7.3	305–336	323	± 4.0	2358	1.61	Norm.
3	9	♂	Cast.	Theelin	C	17	+ 7.4	6.3	163–206	182	± 5.3	1148	1.01	St.
	10	♂	Cast.	Theelin	D	17	− 14.6	4.8	105–161	133	± 2.4	639	0.67	St.
	11	♂	Cast.	Progest.	C	17	+ 7.9	7.5	292–352	319	± 5.4	2394	1.59	Norm.
	12	♂	Cast.	Progest.	D	17	− 23.1	7.5	297–315	305	± 2.1	2291	1.47	Norm.
4	13	♀	Norm.	.....	C	17	+ 15.9	8.2	271–340	312	± 7.5	2522	1.68	Norm.
	14	♀	Norm.	.....	D	17	23.5	3.8	70–127	102	± 7.3	387	0.67	St.
	15	♀	Spd.	.....	C	17	+ 18.4	7.8	283–333	311	± 6.0	2239	1.71	Norm.
	16	♀	Spd.	.....	D	17	− 38.7	7.0	144–248	182	± 10.1	1275	1.09	St.
5	17	♀	Norm.	Theelin	D	17	− 20.6	7.9	232–295	267	± 6.8	2070	1.31	P.st.
	18	♀	Norm.	Stilb.	D	17	− 29.9	4.3	149–171	161	± 2.7	693	0.81	St.
	19	♀	Norm.	Progest.	D	17	− 22.5	6.9	145–199	168	± 6.3	1150	0.86	St.
	20	♀	Spd.	Progest.	D	17	− 14.4	7.0	256–306	282	± 6.4	1970	1.39	P.st.
6	21	♀	Norm.	M.test.	D	17	− 16.5	5.3	140–169	155	± 3.8	819	0.79	St.
	22	♀	Norm.	Test.p.	D	17	17.9	5.8	176–251	211	± 8.7	1222	1.12	St.
	23	♀	Spd.	Test.p.	C	17	+ 22.8	8.0	289–334	315	± 5.1	2510	1.71	Norm.
	24	♀	Spd.	Test.p.	D	17	− 20.5	8.3	141–179	164	± 5.9	1363	1.03	St.
7	25	♀	Norm.	.....	C	7	+ 15.8	8.7	308–309	309	± 0.28	2678	1.51	Norm.
	26	♀	Norm.	.....	No G	7	4.5	5.8	143–185	157	± 5.1	1075	0.76	St.
	27	♀	Preg.	.....	C	7	+ 10.9	8.0	292–308	305	± 2.1	2418	1.51	Norm.
	28	♀	Preg.	.....	No G	7	− 2.9	6.7	286–341	308	± 6.5	2442	1.50	Norm.
8	29	♂ ♀	Im.	.....	C	4	+ 79.1	4.3*	211–285	246	± 7.3	1058	1.31	P.st.
	30	♂ ♀	Im.	.....	D	4	+ 15.2	3.3*	226–256	241	± 3.5	793	1.29	P.st.
	31	♂ ♀	Im.	Test.p.	C	4	+ 116.9	4.5*	372–415	390	± 4.8	1755	1.92	Norm.
	32	♂ ♀	Im.	.....	C	4	+ 98.9	4.2*	369–382	376	± 1.5	1566	1.91	Norm.
	33	♂ ♀	Im.	.....	D	4	+ 9.1	3.0*	154–227	195	± 6.7	585	1.05	St.
	34	♀	Im.	Theelin	C	4	+ 76.6	4.2*	351–380	364	± 2.9	1519	1.81	Norm.

Abbreviations: Cast., castrated; Norm., normal; Spd., spayed; Preg., pregnant; Im., immature; Test.p., testosterone proprionate injd.; M.test., methyl testosterone orally; Progest., progesterone; Stilb., stilbestrol; C, complete diet; D, vitamin-deficient diet; No G, diet deficient in G complex; St., stunted; P.st., partially stunted.

* Only 5 cysticercoids given per rat in groups 29–34.

placed on a complete diet and groups 2 and 4 on a vitamin-deficient diet; 17 days later all 24 rats were infected with 10 cystercercoids each, and autopsied 14 days subsequently. Examination of the results of this experiment in Table 1 will show that, as had previously been demonstrated by Chandler (1943), the worms grew normally in the normal male animals on either a complete or a vitamin-deficient diet. In the castrated animals, however, the growth of the worms was stunted to the same extent as in female hosts on a deficient diet (see Addis and Chandler, 1946), *not only in the animals kept on a deficient diet, but also in the animals given a complete diet!* This suggested the possibility that the male sex hormone (testosterone) is necessary for normal growth of the worms in male rats, regardless of the diet of the hosts. It is interesting to note that castration of male rats, like a deficient diet in female rats, causes some reduction in the number of worms established, though the effect is not as marked (compare groups 1 and 2 with group 14 in Table 1).

Experiment 2

Castrated Males Treated with Testosterone

In this experiment 24 male rats were castrated and divided into four groups (5 to 8, Table 1) of 6 rats each. After recovery, groups 5 and 6 were given 2 mg of testosterone propionate per rat by subcutaneous injection every other day for a total of 18 injections. Groups 7 and 8 were given 4 mg of methyl testosterone per rat by stomach tube every other day for a total of 18 doses. The rats were started on the administration of the hormone far enough ahead of the administration of the cysticercoids so that the treatment would continue through the period of the growth of the worms, up to the time of autopsy. Groups 5 and 7 were placed on a complete diet, and groups 6 and 8 on a vitamin-deficient diet, 17 days prior to giving the cysticercoids.

As will be seen in Table 1, the growth of the worms was normal in all four groups of rats, indicating that testosterone, given either by injection or by mouth, in hosts on either a complete or a vitamin-deficient diet, enables the tapeworms to grow in a normal manner, just as they do in normal male rats. It is also of interest to note that injection of testosterone into castrated rats enables a normal percentage of worms to establish themselves, entirely overcoming the lowering in number of worms established which lack of the fat-soluble vitamins consistently causes in female rats on a deficient diet, or in castrated males not given testosterone.

Experiment 3

Castrated Males Treated with Female Sex Hormones

This experiment was planned to determine the effect of female sex hormones on the growth of tapeworms in castrated male rats. Twenty-four castrated rats were divided into four groups (9 to 12, Table 1). The rats of groups 9 and 10 were given subcutaneous injections of 0.008 mg of theelin (about 25 rat units) every other day for a total of 18 injections, and those of groups 11 and 12 were given similar injections of 0.5 mg of progesterone. Seventeen days prior to infection, groups 9 and 11 were placed on a complete diet and groups 10 and 12 on a vitamin-deficient diet.

As shown in Table 1, the theelin administration failed to prevent the stunting of the growth of tapeworms in castrated male rats, but injections of progesterone appeared to be as effective as those of testosterone. This is interesting in view of the

similar chemical and biological properties of these two hormones. Progesterone also functioned in a manner similar to testosterone in permitting a normal number of worms to be established, whereas theelin had no such effect.

Experiment 4

Normal and Spayed Females

Twenty-four female rats were selected and twelve of them spayed. After recovery of the spayed animals, the rats were divided into four groups (13 to 16, Table 1). Groups 13 and 14 consisted of normal females and groups 15 and 16 of spayed females. Groups 13 and 15 were placed on a complete diet and groups 14 and 16 on a vitamin-deficient diet 17 days prior to infection.

The results of this experiment, shown in Table 1, again demonstrate that a vitamin-deficient diet in normal female rats causes a stunting in the growth of the worms and also a marked reduction in the percentage that succeed in establishing themselves. As far as growth of the worms is concerned, the behavior in the spayed females was similar to that in normal females, since normal growth occurred in the animals on a complete diet, whereas stunting occurred in the animals on a deficient diet. The lowering in establishment of the worms, however, so consistently found in normal female rats on a vitamin-deficient diet, did not occur in the spayed females.

Experiment 5

Normal and Spayed Female Rats Injected with Female Sex Hormones

It is known that a vitamin-deficient diet causes anoestrus in normal female rats, indicating that the normal secretion of the sex hormones is inhibited. In this experiment, therefore, the female sex hormones were supplied to normal and spayed female rats on a vitamin-deficient diet. Four groups of rats (17 to 20, Table 1) were used in this experiment and placed on a vitamin-deficient diet 17 days prior to infection. Groups 17, 18, and 19 consisted of normal females and group 20 of spayed animals. Group 17 received a total of 18 subcutaneous injections of 0.008 mg of theelin per rat, given every other day up to the time of autopsy. The rats of group 18 received a similar number of doses of 0.25 mg diethyl-stilbestrol. The rats of groups 19 and 20 received a similar number of injections of 0.5 mg of progesterone.

It will be seen in Table 1 that in groups 18 and 19, where normal female rats on a vitamin-deficient diet were given stilbestrol and progesterone, respectively, the stunting effect on the growth of the worms was not alleviated. The injection of theelin into normal female rats and of progesterone into spayed rats, also on deficient diets, produced a significant increase in the growth of the worms, though it did not bring it up to that in either normal or spayed rats on complete diets (compare groups 17 and 20 with groups 13 and 15 in Table 1). The reduction in length and width of the worms is only in the neighborhood of 10%, as compared with 40% to 50% in normal females on a deficient diet. The better action of progesterone in spayed than in normal females may be due to the antagonistic effect of theelin in the latter, but it is peculiar that this hormone had less effect in promoting growth in the spayed females than in castrated males. In all of the groups in this experiment the establishment of the worms was greatly increased over that found in normal females on a deficient diet except in group 18, in which stilbestrol was given by mouth.

Experiment 6

Normal and Spayed Females Treated with Testosterone

In this experiment two groups of six normal female rats (21 and 22, Table 1) and two groups of spayed females (23 and 24, Table 1) were given testosterone. Both groups of normal females were kept on a deficient diet; one group (21) was given methyl testosterone by mouth, while the other (22) was given testosterone propionate by injection, the doses being the same as described for male rats in Experiment 2. The two groups of spayed rats were given injections of testosterone propionate by injection; group 23 was given a complete diet, group 24 a deficient diet. All four groups were kept on their respective diets for 17 days prior to infection.

As seen in Table 1, testosterone either by mouth or by injection fails to bring about normal growth of the worms in either normal or spayed female rats on a vitamin-deficient diet. The growth was normal in the spayed rats given testosterone and kept on a complete diet, just as it was in untreated spayed rats. It will be seen that again the number of worms established in the spayed rats on a vitamin-deficient diet was equivalent to that of control rats on a complete diet, while in the normal rats the lack of vitamins markedly reduced the percentage established.

Experiment 7

Pregnant Rats

Because of the failure to bring about normal growth of tapeworms in female rats on a vitamin-deficient diet by the administration of male or female hormones it seemed desirable to study the effect of pregnancy on the growth of the worms. In this experiment 30 female rats were placed with males, and a week later were fed cysticercoids. Half of the rats were placed on a complete diet and half on a diet deficient in the G complex, but with B_1 and the fat-soluble vitamins (A, D, and E) provided in order to maintain pregnancy over the 14-day period before autopsy. Four groups (25 to 28) of 6 rats each were selected for autopsy. Groups 25 and 26 were control non-pregnant rats on complete and vitamin-G-deficient diets, respectively, whereas groups 27 and 28 were pregnant rats on complete and G-deficient diets, respectively. As seen in Table 1 the results in the non-pregnant rats were as expected—normal growth in the rats on a complete diet and stunted growth in those on the G-deficient diet. In both groups of pregnant rats, however, the growth was normal. This is the only instance in which fully normal growth of the worms was obtained in female rats on a G-deficient diet; the effect was fully comparable to the injection of testosterone into castrated male rats.

Experiment 8

Sexually Immature Rats

To study the effect on tapeworm growth in sexually immature rats, young animals between 5 and 6 weeks old, and weighing between 50 and 100 gm, were used. Six groups (29–34) of six rats each, three of young males and three of young females, were selected. Groups 29 and 30 were young males on complete and vitamin-deficient diets, respectively, and group 31 consisted of young males on a complete diet but with 2 mg of testosterone propionate per rat injected subcutaneously every other day throughout the course of the experiment. Groups 32 and 33 were young females

on complete and vitamin-deficient diets, respectively, and group 34 consisted of young females on a complete diet with 0.008 mg of theelin and 0.5 mg of progesterone per rat injected subcutaneously every other day throughout the course of the experiment. Due to the age and weight of the rats, only 5 cysticercoids were fed to each rat 4 days after being placed on their diets.

The results, as shown in Table 1, again demonstrated the need for testosterone in male rats in order to obtain normal tapeworm growth, whether on a complete or deficient diet. In immature males with testosterone injected, the worms averaged 390 mm in length and 1.92 mm in width, the larger size than in other experiments being due to the smaller number of worms per rat. In immature males on either a complete or deficient diet the growth was reduced by about 38%, nearly as much as in castrated adults (44%).

In immature females on complete and deficient diets the behavior was the same as in adult females—normal growth on a complete diet and stunted growth on a deficient diet, whether or not the female sex hormones were injected.

DISCUSSION AND CONCLUSIONS

The fact that the growth of *Hymenolepis diminuta* is normal in male rats on a vitamin deficient diet while it is stunted in female rats on the same diet suggests some relationship between the sex hormones of the host and the vitamin G complex which affects the normal growth of the tapeworms.

The present work shows that castration of male rats causes a stunting in the growth of worms, even in rats maintained on a complete diet, and that the growth of the worms can be brought back to normal in castrated rats by testosterone given either by subcutaneous injections or by mouth. Also, in sexually immature male rats on a complete diet or a vitamin-deficient diet the growth of the worms is inhibited as it is in the castrates, but can be restored to normal by the injection of testosterone. From these observations it is a logical deduction that the male sex hormone is necessary in the host for normal growth of the worms in the male rats. Just how the growth of the worms is affected by the hormone cannot be answered at the present.

The injection of progesterone into castrated males also permits normal growth of the worms. This might be explained by the fact that progesterone and testosterone are chemically and biologically related. Testosterone injected into spayed females causes progestational changes in the uterus. The injection of theelin into castrates failed to bring about a normal growth of the worms.

Spaying a female rat had no effect on the growth of the worms as long as the host was maintained on a complete diet, but the growth of the worms was stunted in spayed as well as normal female rats kept on a vitamin-deficient diet. Also, the growth of the worms was normal in sexually immature females with or without injections of theelin and progesterone as long as the host was on a complete diet.

Injections of theelin, progesterone or testosterone, or oral administration of testosterone or stilbestrol, failed to bring about a normal growth of the worms in normal female rats on a vitamin-deficient diet. Injections of testosterone or progesterone into spayed rats on a vitamin-deficient diet also failed to bring about a growth of the worms comparable to that in rats on a complete diet, although the progesterone did cause an increase in the growth over that in untreated female rats on a vitamin-deficient diet.

Only in pregnant rats have we been able to obtain a normal growth of the worms in female rats on a diet deficient in the G complex. It is possible that this can be attributed to the gonadotropic hormone found in the urine of pregnant animals. The fact that this hormone is very similar to the gonadotropic hormone of the pituitary suggests that some hormone secreted by the pituitary is necessary for the normal growth of the worms.

It is known that a diet deficient in the B complex brings about a stage of anoestrus in the female rat with ultimate cessation of sex hormone production, and further that a much greater depletion over a longer period of time is necessary in the male than in the female before a decrease in sex hormone production occurs. This might explain the fact that a vitamin-deficient diet does not cause a stunting in the growth of the worms in the male rats while it does in the female, if, as appears to be true, the worms require testosterone or a chemically related substance in the host for normal growth. This inhibition of production of the sex hormones in animals on a vitamin-deficient diet is believed to be due to the failure of secretion of gonadotropic hormones by the pituitary, and not to any effect on the gonads directly. This again indicates that the pituitary might be involved in the normal growth of the worms.

At the present the only definite conclusion that can be reached is that the worms are dependent upon testosterone for normal growth in male rats, and that progesterone can be substituted for testosterone. Nothing definite can be said at the present about the relation between the growth of the worms and the sex hormones in female rats, other than that pregnancy supplies the factor needed by the worms for normal growth when the host is on a G-complex-deficient diet.

SUGGESTIONS FOR FURTHER INVESTIGATIONS

The results of these experiments on the relation between the sex hormones and the growth of the worms have suggested several interesting problems. It would be of interest to make bio-assays to determine the testosterone potency of extracts made from worms taken from normal male rats, castrated rats, and castrated rats with testosterone injected. This might show more clearly how this hormone is utilized by the worm and whether or not absorption of the hormone occurs from the mucous membrane of the host by the worms.

In the work with female rats it would be desirable to study the effect of pituitary injections upon the growth of the worms. Also, the injection of pregnant urine into normal female rats on a vitamin-deficient diet might throw some light on the picture.

Probably the most helpful of any investigation would be the development of a satisfactory method of rearing tapeworms *in vitro*.

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FURTHER STUDIES ON THE VITAMIN REQUIREMENT OF TAPEWORMS

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In an earlier paper (1944) we corroborated and extended the work of one of us (Chandler, 1943) showing that *Hymenolepis diminuta* grows in a normal manner in the absence of any vitamins in the diet of the host except something associated with the G complex, absence of which causes a marked stunting in growth in female but not in male rats. This stunting becomes intensified as the depletion period in the host prior to infection is increased.

In the present paper are reported a series of experiments designed to determine, if possible, what factor or factors in the G complex is responsible for the stunting effect. The hosts used were female white rats three to four months old, weighing between 150 and 200 gm at the beginning of the experiments. The rats were infected by oral administration of artificially reared cysticercoids as described in our earlier paper. The complete diet for control animals was the same as described in that paper. The basic vitamin-deficient diet was also the same except that the fat-soluble vitamins were added in the manner previously described, for the benefit of the host animals. The G complex, when used, was provided in the form of 9 gm of autoclaved brewer's yeast per rat per day. Other vitamins or nutritional factors, added in synthetic or concentrated form, were dissolved in 20% alcohol, and the proper amount added to the basic diet each day.

Riboflavin (B_2), pyridoxine hydrochloride (B_6), calcium pantothenate, nicotinic acid, p-aminobenzoic acid, inositol, and choline were used in pure synthetic form and provided at the rate of 100 micrograms per day per rat. Biotin was used in the form of a concentrate containing 20 micrograms per cc, and provided at the rate of 30 micrograms per day per rat. Folic acid was provided in the form of a concentrate at the rate of 90 micrograms per day per rat.

EXPERIMENT I

Fifteen groups of 6 female rats each were placed on diets as follows: Groups 1, 2, and 3, complete diet (repeated because the experiments were run in three lots at different times); Groups 4 and 5, a diet deficient in B_1 and the entire G complex; Groups 6 to 15 inclusive, the basic diet deficient in B_1 and the G complex, but with nicotinic acid added to group 6, pyridoxine hydrochloride to group 7, calcium pantothenate to group 8, riboflavin to group 9, biotin concentrate to group 10, p-aminobenzoic acid to group 11, inositol to group 12, choline to group 13, folic acid concentrate to group 14, and all of the above-named components of the G complex to group 15. All of the groups were kept on the experimental diet for 4 days prior to infection as well as during the 14 days allowed for growth of the worms prior to autopsy, except control groups 3 and 5, and experimental groups 10 and 11, which were kept on their respective diets for 17 days prior to infection.

The upper part of Table 1 shows the results of these experiments. It will be seen by comparison of the length and width of the worms kept on a complete diet (groups

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TABLE 1.—*Effect of vitamin-deficient diets on the growth of* Hymenolepis diminuta

Diet	Days on diet before inf.	Change in wt. of rats (per cent)	Av. No. of worms at autopsy	Length of worms (mm)				Av. width of worms (mm)
				Range of av. in indiv. rats	Av. per worm	Prob. error of av.	Total per rat	
Experiment I								
1. Complete	4	+16.0	7.8	304–471	350	±16	2,729	1.58
2. Complete	4	+15.0	8.0	358–380	368	± 8.5	2,948	1.73
3. Complete	17	+18.1	8.0	294–386	350	± 8.8	2,791	1.62
4. No B1 or G complex	4	− 8.7	6.8	170–246	199	± 9.8	1,378	1.02
5. No B1 or G Complex	17	−18.2	5.5	110–254	195	±15.3	1,073	0.93
6. Nicotinic acid	4	− 6.5	8.1	127–226	177	±10.1	1,436	0.94
7. Pyridoxine	4	− 6.5	4.7	113–282	193	±23.9	908	0.99
8. Panthothenic acid	4	− 6.0	6.2	135–253	176	±16.3	1,093	0.96
9. Riboflavin	4	+ 8.5	6.7	143–250	209	±12.1	1,400	1.00
10. Biotin conc.	17	−18.1	7.2	83–178	118	± 8.7	850	0.81
11. p-Aminobenzoic acid	17	−15.0	5.7	83–241	175	±21.2	997	0.87
12. Inositol	4	+ 6.3	7.3	209–248	228	± 4.6	1,664	1.15
13. Choline	4	+ 3.3	7.2	171–236	192	± 8.7	1,381	1.02
14. Folic acid conc.	4	− 3.4	7.7	107–195	151	± 8.6	1,166	0.94
15. All synthetic vit.	4	+ 7.6	6.2	127–203	185	± 6.7	1,146	0.88
Experiment II								
16. Yeast concentrate	4	+14.6	7.2	127–201	177	± 7.4	1,276	1.08
17. Liver extract, to stomach	4	+ 2.7	6.3	246–311	277	± 6.6	1,743	1.23
18. Liver extract and yeast concentrate	4	+24.7	7.7	261–308	278	± 4.6	2,145	1.34
19. Liver extract in food	4	+ 3.2	7.7	252–319	294	± 6.4	2,267	1.51
20. Liver extract and inositol in food	4	+ 4.9	8.2	254–358	283	±10.6	2,500	1.35

1, 2, and 3) with those kept on a diet deficient in B_1 and the G complex (groups 4 and 5) that the absence of these vitamins causes a reduction of about 45% in the length of the worms, and of over 40% in their width. It will also be seen that none of the synthetic vitamins, or all of them collectively, has any significant effect in preventing this stunting effect. The average reductions in length in rats in the different groups to which synthetic vitamins or vitamin concentrates were given amounted to from 36% to 58% except in the group to which biotin concentrate was given. In this group the reduction was 67%, suggesting that possibly the addition of this vitamin somehow intensified the stunting action of the vitamin deficiency, either by an effect on the host or on the worms. The number of worms established was apparently not affected except in the case of pyridoxine hydrochloride. In this group the average number of worms per rat (4.7) was distinctly lower than in any other groups kept on their experimental diets for only 4 days prior to infection. Whether or not this difference is significant is questionable without repetition of the experiment.

EXPERIMENT II

Since it became evident from Experiment I that the factor or factors required by *Hymenolepis diminuta* in female white rats, although present in autoclaved brewer's yeast, was absent in the known components of the G complex, further experiments were performed in an effort to determine its nature or at least its occurrence. A yeast concentrate obtained through the courtesy of the Fleischmann Laboratories and a liver extract prepared by the Eli Lilly Company were experimented with. The yeast extract was stated to contain: thiamin, 0.6 to 0.9 mg/gm; calcium pantothenate, 0.5 mg/gm; riboflavin, 0.2 mg/gm; niacin, 1.7 to 2.0 mg/gm; pyridoxine, 0.1 mg/gm; inositol, 2.98 mg/gm; biotin, 0.0012 mg/gm; choline, 2.3 to 6.85 mg/gm; and folic acid, 30 to 40% of the value of Elvehjem's standard liver

extract. No complete analysis of the liver extract was obtainable, but it was a product processed for its anti-anemia potency, and was stated to contain the G complex.

Five groups of 6 female rats each were treated daily as follows: Group 16, 1 gm of yeast concentrate in the food of each rat; Group 17, 0.1 cc of liver extract given by stomach tube; group 18, 1 gm yeast concentrate in the food and 0.1 cc liver extract by stomach tube; group 19, 0.1 cc liver extract mixed with the food; and group 20, 0.1 cc liver extract plus 100 mg inositol mixed with the food. The inositol was added since addition of this factor had caused a slight but probably insignificant decrease in the stunting effect of a G-deficient diet in Experiment I.

The results of this experiment are shown in the lower part of Table 1. It will be seen that none of these diets brought the growth of the worms up to normal. The yeast concentrate was entirely ineffective in overcoming the stunting effect of a G-deficient diet. The liver extract, however, in all four of the groups in which it was used, reduced the stunting effect by about half, from around 45% to little more than 20%. It seems evident, therefore, that liver extract contains some factor necessary for normal growth of tapeworms in female rats which is not present in the yeast concentrate, but since the growth of the worms did not come up to that of the worms in rats fed autoclaved brewer's yeast, it may be concluded either that this necessary factor is present in inadequate amount, or that some additional factor is lacking.

Since it had been expected that the yeast concentrate would contain the necessary food factor, and it seemed evident that it had been destroyed by some step in its preparation in the Fleischmann Laboratories, we prepared a hydrolyzed brewer's yeast filtrate, under known conditions of preparation, in our own laboratory. We mixed 25 gm of yeast with 500 cc of water and hydrolyzed it at pH 5.0 at 82° C for 15 or 20 minutes. The mixture was filtered and the filtrate dried. Each of six female rats was given 1 gm of the dry filtrate in an otherwise G-deficient diet, beginning 4 days before infection with 10 cysticercoids. At autopsy only three of the rats were infected, each with two worms, but the range in length of the worms present was 438 to 633 mm, average 516.7 mm, which is the normal growth that would be expected for this number of worms in rats on a fully adequate diet. Although it is evident that something was wrong with the cysticercoids or their administration to bring about such a small percentage of establishment, the normal growth of the worms that were established suggests that the necessary factor was present in our hydrolyzed yeast extract. This, however, should be confirmed before a definite conclusion can be reached.

DISCUSSIONS AND CONCLUSIONS

Work done on the vitamin requirement of *Hymenolepis diminuta* in rats (Hager, 1941; Chandler, 1943; and Addis and Chandler, 1944) at the present shows that the worms are independent of vitamin B_1 in the diet of the host for normal establishment and growth, independent of vitamins A, D, and E for growth but dependent on the fat-soluble vitamins for normal establishment, and dependent on some factor or factors found in brewer's yeast for normal establishment and growth.

The necessary yeast component apparently is not any of the eight known components of the G complex, since the worms failed to grow normally in rats given

these vitamins either singly or in combination in the synthetic form and also since the growth of the worms was stunted in the rats given Fleischmann's yeast concentrate. The experiment in which a hydrolyzed yeast product prepared in our own laboratory was used suggests that the necessary substance was present in it, but, as noted above, the results of this experiment require confirmation before they can be accepted. The factor is apparently heat-stable since the worms grow normally in rats when autoclaved yeast is added to a vitamin-deficient diet.

Liver extract reduces the stunting effect of a G-deficient diet by about one-half, from which it may be concluded either that the necessary factor is present in this product in inadequate amount, or that more than one factor is involved, not all of which are present in the extract. The factor or factors involved may be one of the other postulated components of the G complex, or some new factor which has heretofore been unknown. Its peculiar relation to the sex hormones of the host animal will be discussed in another paper.

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IN MEMORIAM

Clarence John Addis, Jr. (1921–1946)

Dr. Clarence J. Addis, Jr., died on July 4, 1946, at the tender age of 24, as the result of a brain tumor that had affected his health for about a year. He was born at Van Buren, Ark., on December 5, 1921. He entered Ouachita College, Arkadelphia, Ark., in September, 1939, and obtained his B.S. degree, *cum laude,* in August, 1942. He was awarded a Fellowship in Biology at Rice Institute that fall, and obtained his M.S. degree in February, 1944, and his Ph.D. degree in June, 1945, from that institution.

Afflicted by a deformed foot, he was unable to join the armed forces. He made his contribution to the war effort, however, in a special research problem on Texas species of *Phlebotomus* and their ability to transmit leishmaniasis, under the sponsorship of the Office of Scientific Research and Development of the National Research Council, for which he was given a special citation in July, 1945.

While at Rice Dr. Addis became interested in the nutrition of tapeworms and wrote his master's and doctor's theses on that subject. During his brief scientific career of only three years he was the author or coauthor of seven parasitological papers, including two which are published posthumously in the present issue of this journal. While at Rice, in addition to his regular graduate work and his special

research for the O.S.R.D., he was a capable and universally liked laboratory assistant in a variety of courses. His cheerful devotion to duty and enthusiastic interest in his work was an inspiration not only to students, but also to the faculty members with whom he worked. After obtaining his Ph.D. degree he accepted a position as research assistant under Dr. Augustine at Harvard, but his unfortunate illness made it impossible for him to undertake his work there. Thus what promised to be a brilliant career came to a tragic end almost at its beginning.

Dr. Addis had all the attributes of a true scientist. He was intellectually keen, sound in judgment, endowed with initiative and resourcefulness, enthusiastic about his work, and industrious to a fault. He was, in addition, endowed with other qualities that not all scientists have. He was deeply religious, loved music and poetry, took an active interest in sports in spite of inability to participate in them and was thoroughly "human" in his relations to other people. He once said, "The more I study and the more I learn, the greater does God, the Creator, become to me." What he accomplished in his short span of life should be an inspiration and a challenge to many another young scientist for years to come.—Asa C. Chandler, *Rice Institute, Houston, Texas.*

THREE NEW *FINLAYAS* OF THE *DISSIMILIS* GROUP (DIPTERA: CULICIDAE) FROM THE PHILIPPINE ISLANDS[1]

LLOYD E. ROZEBOOM[2]

Aedes dissimilis was described by Leicester (1908) from specimens taken from water in the hollow of a tree in the jungle at Ampang, Federated Malay States. Barraud (1924) described the female and male from India, and added the variety *karwari,* which differs from the type in having a golden-scaled scutum in the female as well as in the male. Later (1934) Barraud listed briefly the characters of the larva.

Recent collections by entomologists on military duty in the Philippine Islands have resulted in the finding of three dark-legged *Finlayas* which are closely related to *dissimilis* but nevertheless are distinct enough to justify their description as new species.

Aedes (*Finlaya*) *paradissimilis,* new species

FEMALE. A small to medium-sized mosquito with dark integument, dark legs and wings, and with patches of silvery scales on the pleurae and abdomen. *Head:* Palpus about one-fifth the length of the proboscis; palpus and proboscis dark-scaled. Vertex covered with broad, flat, black scales; usually with a small patch of broad, flat, white scales in the middle at the front; a pale border along the eyes to a lateral pale area; forked scales on nape black. *Thorax:* Prothoracic lobes with some flat pale scales below and several long black bristles on the anterior margin; another patch of broad, flat, pale scales and a long bristle on the propleuron. Posterior pronotum clothed with broad, flat, silvery scales. Scutum black, clothed with narrow, purple-black scales, sometimes with a narrow central stripe of golden scales; long black bristles at anterior margin, above the wing base, and before the scutellum. Each scutellar lobe with a patch of flat, black scales; bristles black. Pleuron with a large patch of broad, flat, silvery scales covering the posterior and upper area of the sternopleuron and the upper portion of the mesopleuron; a patch of similar scales before the wing base. Coxae with silvery flat scales on antero-lateral surface. Trochanters yellow, with white scales below. Fore femur dark, sometimes with a few pale scales at base on the inner-anterior surface and occasionally with a few ventral pale scales at apex; mid femur with an apical white spot on ventral and anterior surface, and a short, linear white spot at middle on anterior surface; hind femur white on the basal half, sometimes with a dark stripe along the outer edge to the base, the apical half dark but with a white spot on the ventral and anterior surfaces at the apex. Tibiae and tarsi dark. Claws of fore and mid tarsi toothed. Wing scales dark. Halter with yellow stem and dark-scaled knob. *Abdomen:* Tergites dark-scaled, with lateral basal white spots which on the posterior segments may extend dorsally to form complete basal or sub-basal bands. Sternites with basal white bands.

MALE. Coloration similar to that of the female. The scutum may be dark, but usually there is a large patch of golden scales on the anterior half. Palpus about three-fourths the length of the proboscis. *Genitalia* (Figs. 1 and 3): Sidepiece between four and five times as long as the width at the base, cylindrical; the outer, dorsal, and ventral surfaces with scales and long bristles; the inner surface with shorter setae. Clasper two-fifths the length of the sidepiece, tapering to the tip; terminal spine about a third the length of the clasper, with a curved point. Claspette a low, thumb-like projection, with a long tapering seta and a shorter, slender, papillated hair at apex and one to three similar hairs on the inner slope. Ninth tergite lobed, each lobe with two to five short, stout setae. Ninth sternite with two to four stout, straight marginal setae and several smaller setae lateral and basal to the marginal ones.

LARVA. *Head* (Figs. 7 and 11): Antenna somewhat shorter than the length of the head, cylindrical, smooth or with one or several very small basal spines; antennal hair single, stout, darkly pigmented, and inserted at or below the middle. Preclypeal spines slender. Frontal

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[1] This is the fifth of a series of papers reporting the results of studies on Philippine mosquitoes by U. S. Naval Medical Research Unit Number 2.

[2] From the Department of Parasitology, the Johns Hopkins School of Hygiene and Public Health.

hairs 4, 5, 6, and 7 inserted posterior to base of antennae; hair 4 small, with 4–6 branches; hairs 5 and 6 long, extending well beyond the labrum, 5 longer than 6, single or occasionally double, smooth or with minute lateral spicules; hair 7 with 7–10 branches; hairs 8 and 9 with 2–4 branches. Subantennal hair 12 with 4–6 branches. Ventral hairs all small; hair 13 with 3–6 branches, 15 with 6–11, 17 with 1–2, 18 with 1, and 20 with 5–8 branches. Mentum (Fig. 12) with large central tooth and 9–13 teeth on each side, the lower ones larger and more widely spaced; the teeth rather blunt at apex. Mandible (Fig. 9) with posterior process bifurcate. *Thorax:* Prothoracic hair O branched at the base into 14–20 branches; prothoracic hairs 1 and 2 long and single; hair 3 with 3–4 branches. Pleural hairs (Fig. 8): Prothoracic pleurals very small, mesothoracic pleurals with a group of three very stout hairs, similar hairs on the metathoracic group less stout. *Abdomen:* Lateral hair 6 (Fig. 10) of segments I and II double, darkly pigmented, and very stout; on segments III–VI these hairs are long, slender, and single. Comb (Fig. 6) with 70–80 teeth in a triangular patch, the central posterior teeth elongate, tapering basally and expanded apically, the apex with a large central tooth and a fringe of progressively smaller spines laterally; other teeth may have a simple apical fringe. Siphon dark brown, about four times the length of the diameter at the base; pecten of 20–25 close-set teeth; each tooth rather short, stout, tapering rather abruptly into a blunt point, and with two to five small denticles at the base; ventral hair tuft with 3–4 branches, inserted just beyond the pecten. Anal plate darkly pigmented, incomplete ventrally; the posterior margin with some short, stout spines; lateral hair short, with 2–5 branches; inner submedian caudal hair (isc) of about 10 branches; outer submedian caudal hair (osc) very long and single; anal gills variable, the dorsal pair about equal to or almost twice the length of the anal plate, the ventral pair shorter than the dorsal gills.

TYPE. *Holotype:* Male, with larval and pupal skins, and mounted genitalia; Cape Melville, Balabac, P. I., June 23, 1945 (D. R. Johnson and Jean Laffoon), reared from tree hole; deposited in U. S. National Museum. *Paratypes:* 3 males and 4 females with associated larval and pupal skins, 27 unassociated larvae, same data as for holotype; 4 unassociated larvae, Irahuan River, Palawan, P. I., June 2, 1945 (D. R. Johnson and Jean Laffoon), from tree holes; 2 males, 2 females, and 3 unassociated larvae, N'golos, Calicoan Island, P. I., December 20–29, 1944 and January 12, 1945 (J. H. Paullus); 4 males, 2 females, and 5 unassociated larvae, Lagolago, Baybay, Leyte, P. I., January 21 and 26, 1945, Libjo, Dinagat, P. I., April 18 and 19, 1945, and Diit River, Tacloban, Leyte, P. I., September 23, 1945 (H. R. Roberts), from tree holes, cut bamboo, and "palm stubs." Paratypes deposited in the U. S. National Museum, the British Museum, the Johns Hopkins University, and the Academy of Natural Sciences of Philadelphia.

Aedes (*Finlaya*) *leucopleurus,* new species

FEMALE. Unknown; probably indistinguishable from *paradissimilis.*

MALE. Coloration similar to that of *paradissimilis.* Some of the forked scales on the nape yellow. Scutum with some yellow scales along mid-line. Palpus two-thirds the length of the proboscis. *Genitalia:* Indistinguishable from those of *paradissimilis.* Claspette (Fig. 2) with a long, tapering seta and a smaller papillated hair at apex, and one or two similar hairs on inner slope.

LARVA. *Head* (Fig. 14): Antenna shorter than the length of the head, cylindrical, with some small basal spines; antennal hair single (in one specimen a hair is split into two branches distally), relatively slender, and inserted near the middle. Frontal hairs 4, 5, 6, and 7 all inserted posterior to base of antenna; hair 4 small, with 13–16 branches; hair 5 long, extending beyond the labrum, with 3–4 branches; (hair 6 absent from all of the five larvae available for study); hair 7 long, with 7–11 branches; hair 8 with 2–3 and hair 9 with 5–8 branches. Subantennal hair 12 with 3–6 branches. Ventral hairs similar to those of the other species; hair 20 with 3–8 branches. Mentum (Fig. 16) with a large central tooth and 10–14 teeth on each side; the lower ones larger, more widely spaced, and sharply pointed. Mandible (Fig. 15) with posterior process truncate. *Thorax*: Prothoracic hair O (Fig. 13) with 40–50 branches; hair 1 long, with 3 branches; hair 2 long, single; hair 3 long, with 6–8 branches. Pleural hairs similar to those of *paradissimilis* except that they are somewhat less stout. *Abdomen:* Lateral hair 6 (Fig. 18) of segment I darkly pigmented, stout, with 2–3 branches; hair 6 of segment II pigmented, with 1–2 branches, and only about one-half the length of hair 6 on segment I; lateral hair 6 of segments III–VI single, rarely double. Comb (Fig. 17) with 40–55 small teeth in a patch, each tooth abruptly tapered below, expanded apically, with a fringe of spines at the apex and smaller ones laterally. Siphon dark brown, about four times as long as the width at the base; pecten of 23–26 close-set teeth; each tooth long, slender on distal half and tapering to a sharp point; two or three small denticles basally, the largest of which is situated towards the middle of the tooth; ventral hair tuft inserted beyond the pecten, with 4–6 branches. Anal plate dark brown, interrupted ventrally, with small spines at posterior margin; lateral hair split

distally into 2–5 branches; isc with 3 or 4 branches, osc long and single; dorsal gills twice the length of the plate, ventral gills about one-third as long as the dorsal pair.

TYPE. *Holotype:* Male, with larval and pupal skins, and mounted genitalia; Irahuan River, Palawan, June 2, 1945 (D. R. Johnson and Jean Laffoon), reared from tree holes; deposited in U. S. National Museum. *Paratypes:* 4 unassociated larvae, Pilapil, Culion Island, P. I., June 20, 1945 (Jean Laffoon), from tree hole. Paratypes deposited in the U. S. National Museum and the British Museum.

Aedes (*Finlaya*) *luzonensis,* new species

FEMALE. Indistinguishable from the female of *A. paradissimilis.* The pale median stripe of the mid femur is longer, about one-half the length of the femur; the apical white spot on the hind femur rings the segment. Basal bands on tergites incomplete. Palpus about one-seventh the length of the proboscis.

MALE. Coloration similar to that of the female; in the three undamaged males available, there was no evidence of yellowish or white scaling on the scutum. Palpus about two-thirds the length of the proboscis. *Genitalia:* Similar to those of *paradissimilis,* but in the four specimens studied, the claspette (Fig. 4) differed in not having the long, tapering seta; instead, there were two or three slender hairs at the apex.

LARVA. *Head* (Fig. 19): Antenna shorter than the length of the head, cylindrical, smooth, or with a few very small spines at base; antennal hair single, less stout than in *paradissimilis,* inserted at or beyond the middle. Preclypeal spines slender. Frontal hairs 4, 5, 6, and 7 all inserted posterior to base of antennae; 4 very small, with 4–8 branches; hairs 5 and 6 single, 5 not reaching or at most extending slightly beyond the labrum, 6 shorter than 5; hair 7 short, with 10–15 branches; hairs 8 and 9 with 2–5 branches. Subantennal hair 12 with 3–8 branches. Ventral hairs all small, similar to those of *paradissimilis.* Mentum with a large central tooth, and 12–13 teeth on each side, the lower ones larger and more widely spaced, the teeth rather blunt. Mandible (Fig. 21) with posterior process bifurcate. *Thorax:* Prothoracic hair O with about 25–35 branches; hair 1 single, 2 with 1–4 branches, and 3 with 3–5 branches. Pleural hairs similar to those of *paradissimilis. Abdomen:* Lateral hair 6 (Fig. 20) of segments I and II not so stout and less heavily pigmented than in *paradissimilis,* with 3–4 branches, those on II a little shorter than those on I; hair 6 on segments III–V split at the base into 2–4 branches. Comb (Fig. 22) with 60–70 teeth in a triangular patch; each tooth tapered basally, the apex expanded and fringed with fine spines, differing from *paradissimilis* in the absence of an enlarged central spine. Siphon dark brown, three or four times as long as the width at the base; pecten of 21–28 close-set teeth; each tooth long, gradually tapering to a sharp point and with 2 or 3 small basal denticles; ventral hair tuft with 4–6 branches, inserted beyond the pecten. Anal plate dark brown, narrowly interrupted ventrally, the posterior margin with some minute spines; lateral hair short, with 2–4 branches; isc of about 6 branches, osc very long and single; anal gills short, tapering to a blunt point.

TYPE. *Holotype:* Male, with larval and pupal skins and mounted genitalia; Subic Bay, Luzon, P. I., July 10, 1945 (M. J. MacMillan), reared from tree hole; deposited in U. S. National Museum. *Paratypes:* 2 males and 5 females, with associated larval and pupal skins; 2 males and 3 females without larval or pupal skins, all collected at Subic Bay, Luzon, between July 6 and 20, 1945, from tree holes and a wooden bucket. Paratypes collected by M. J. MacMillan, E. S. Zolick, and L. E. Rozeboom. Paratypes deposited in the U. S. National Museum, the British Museum, and the Johns Hopkins University.

Taxonomic Discussion.—Edwards (1932) placed *dissimilis* in his Group H of the *Finlayas,* which have completely dark hind tarsi. Barraud (1924) pointed out that *dissimilis* is set apart from the other groups of Indian *Finlayas* by the dark proboscis, tibiae, tarsi, and scutum (in the typical female); by the median silvery mark on the mid femur, and by the male genitalia. In as much as the females of the three Philippine species appear to be indistinguishable from *dissimilis,* it is evident that there is a closely-related group of species separable from the other *Finlayas* by the characters listed above, and distinguishable from one another principally in the larval stage.

The only specimens of *dissimilis* available for comparison were a female and a male in the U. S. National Museum, from Sukna, Northern Bengal, India, referred to by Barraud (1924) as the type form. The claspette of the male is shown in Fig. 5, and agrees with the illustrations of Barraud (1924, 1934) in that the papillated hairs

(p) at the apex and on the slope are quite small. In *paradissimilis* and *leucopleurus* these hairs are longer and stouter.

A. luzonensis is separable by the presence of two or three hairs of about equal size at the apex of the claspette; *dissimilis, paradissimilis,* and *leucopleurus* have a single large seta and two or three smaller papillated hairs on the claspette. The larva differs from those of *leucopleurus* and *paradissimilis* in the small, single head hairs 5 and 6, the multiple-branched and relatively slender lateral hair 6 on abdominal segments I and II, the branched hair 6 on segments III to V, and the structure of the comb and pecten teeth.

A. leucopleurus is readily distinguished from the other three species by the branched frontal head hair 5 of the larva. Other differences between this species and the larvae of *paradissimilis* and *luzonensis* are found especially in the branching of head hairs 4 and 9, the branching of the prothoracic submedian hairs, the shape of the posterior process of the mandible, the structure of the comb and pecten teeth, and lateral hair 6 of abdominal segments I and II.

Of the three Philippine species, *A. paradissimilis* appears to be the most closely related to typical *dissimilis*. The difference in the size of the claspette hairs may be of minor importance; however, Barraud (1934) stated that in *dissimilis* larvae the lateral hairs of the thorax and abdomen are moderately developed, whereas in *paradissimilis* the most striking feature of the larva is the very stout, darkly pigmented lateral hair of abdominal segments I and II; furthermore, there are stout, spine-like hairs in the meso- and metathoracic pleural groups. Barraud made no mention of an enlarged central spine in the apical fringe of the comb teeth, and described the saddle as being without spines on the border. For these reasons, *paradissimilis* is considered to be a different species than *dissimilis*.

Barraud (1924) established the variety *karwari* on the golden-scaled scutum of the female. It is concluded that the golden scales noted on the scutum of some females of *paradissimilis* have no taxonomic significance.

The position of *A. leucomeres* Giles (1904) merits some comment. Bohart (1944) stated that although no specimens are in the U. S. National Museum, the scutal markings described by Giles are distinct. These markings consist of two anterior spots, a median line, and some white markings posteriorly. However, Edwards (1922) resorted to the width of the pale band around the eyes to separate *leucomeres* from *dissimilis,* which would lead one to suspect that Edwards failed to confirm the presence of distinctive scutal markings in *leucomeres* upon reexamination of Giles' material. A similar misconception could result from Leicester's description of *dissimilis,* by the statement that the mesonotum has "a large quadrangular patch of broad pearly-white scales inserted on the shoulders." These "shoulders" evidently are the posterior pronota. Perhaps the relationship of *leucomeres* to *dissimilis* could be determined by comparison with Giles' type; however, as Theobald (1910) pointed out, the description is of little value, and the type is mostly denuded, so that it would seem to be impossible to establish the true identity of *leucomeres*.

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EXPLANATION OF PLATES

PLATE I

FIG. 1. *A. paradissimilis.* Male genitalia.
FIG. 2. *A. leucopleurus.* Claspette of male genitalia.
FIG. 3. *A. paradissimilis.* Claspette of male genitalia.
FIG. 4. *A. luzonensis.* Claspette of male genitalia.
FIG. 5. *A. dissimilis* (Sukna, India). Claspette of male genitalia.
FIG. 6. *A. paradissimilis.* Terminal abdominal segments of larva.

PLATE II

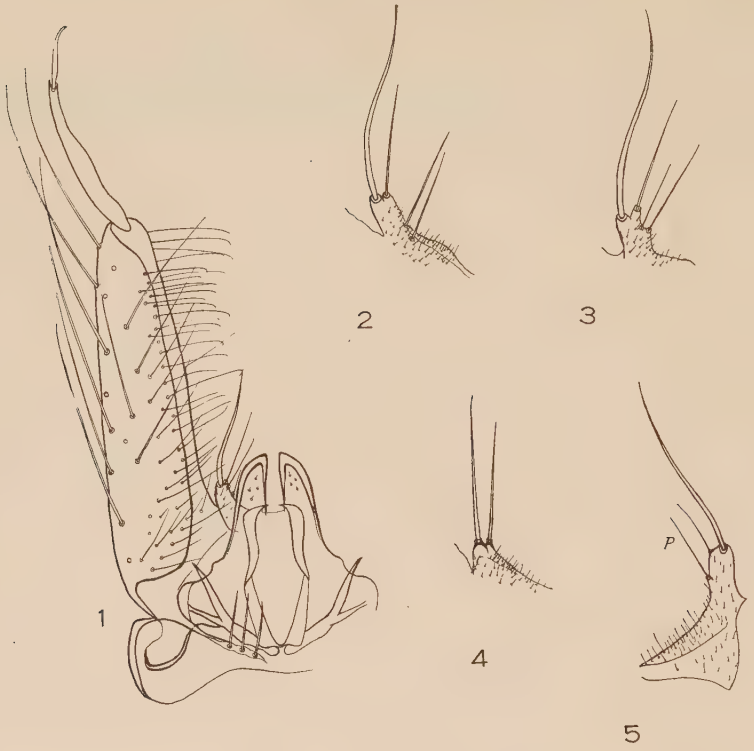
FIG. 7. *A. paradissimilis.* Dorsal aspect of larval head.
FIG. 8. " Thoracic pleural hairs of larva.
FIG. 9. " Mandible of larva.
FIG. 10. " Hair 6 of abdominal segments I and II of larva.
FIG. 11. " Ventral aspect of head of larva.
FIG. 12. " Mentum of larva.

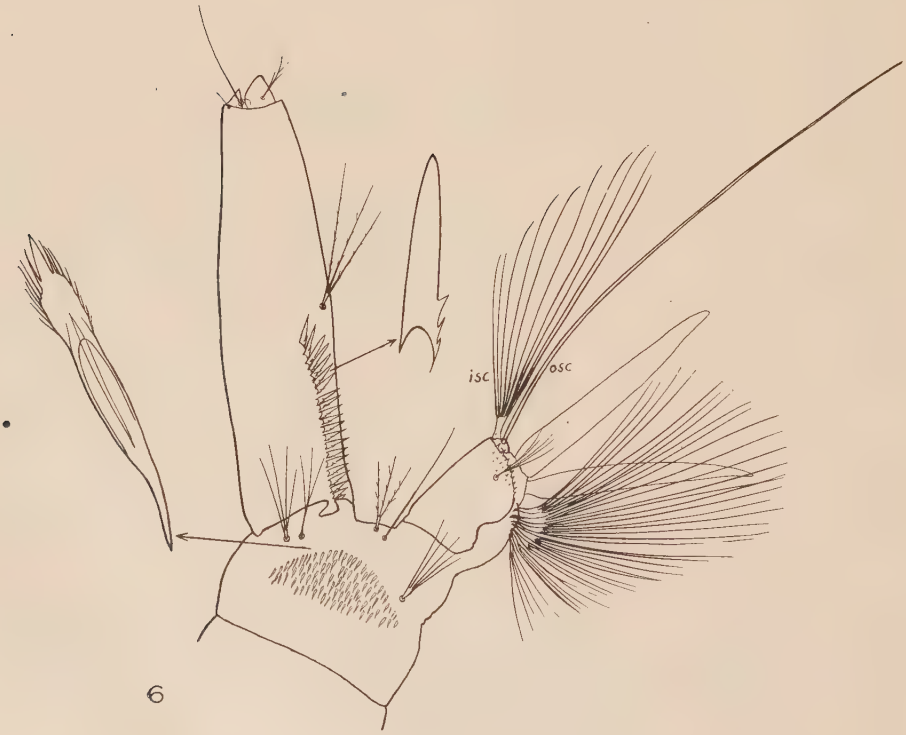
PLATE III

FIG. 13. *A. leucopleurus.* Submedian dorsal prothoracic hairs of larva.
FIG. 14. " Dorsal aspect of head of larva.
FIG. 15. " Mandible of larva.
FIG. 16. " Mentum of larva.
FIG. 17. " Terminal abdominal segments of larva.
FIG. 18. " Hair 6 of abdominal segments I and II of larva.

PLATE IV

FIG. 19. *A. luzonensis.* Dorsal aspect of head of larva.
FIG. 20. " Hair 6 of abdominal segments I and II of larva.
FIG. 21. " Mandible of larva.
FIG. 22. " Terminal abdominal segments of larva.





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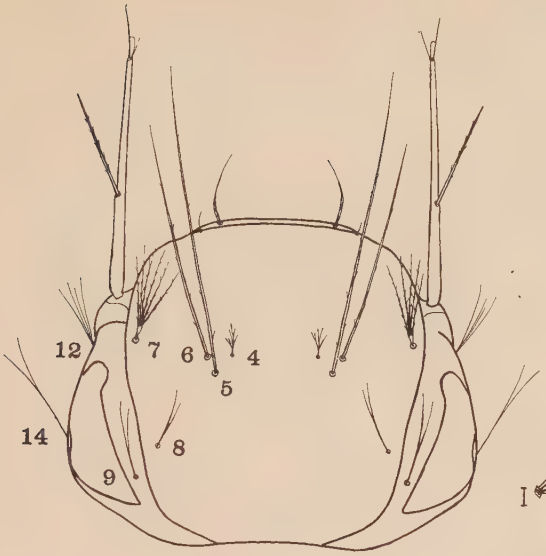

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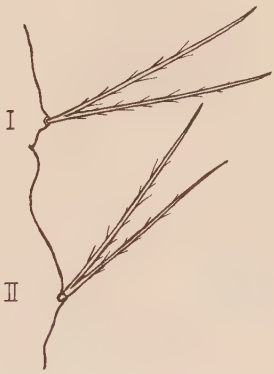

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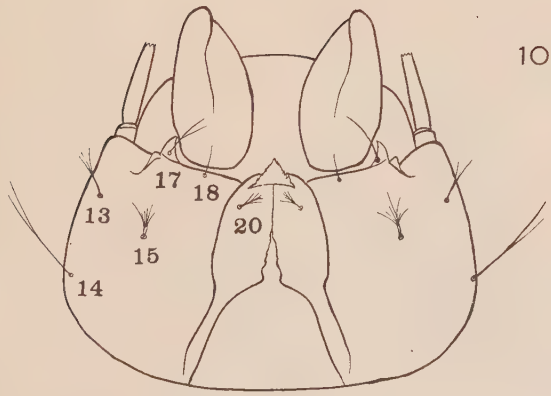

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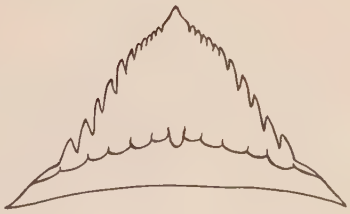

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PLATE II

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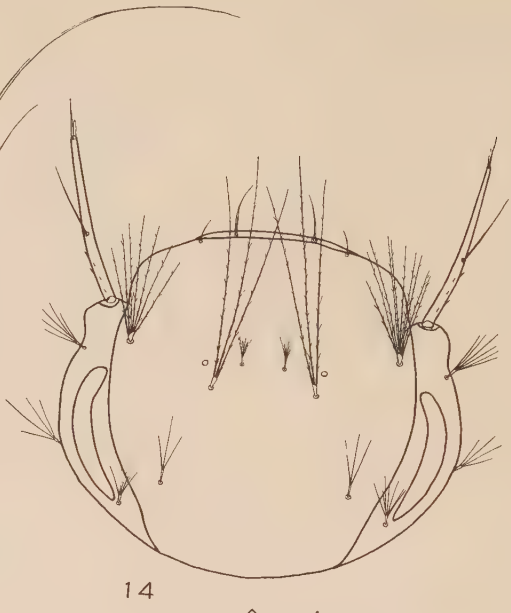

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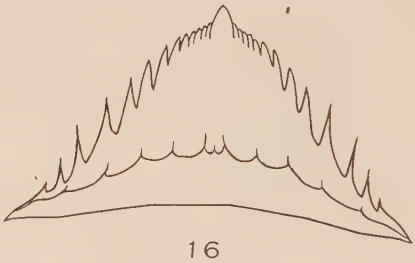

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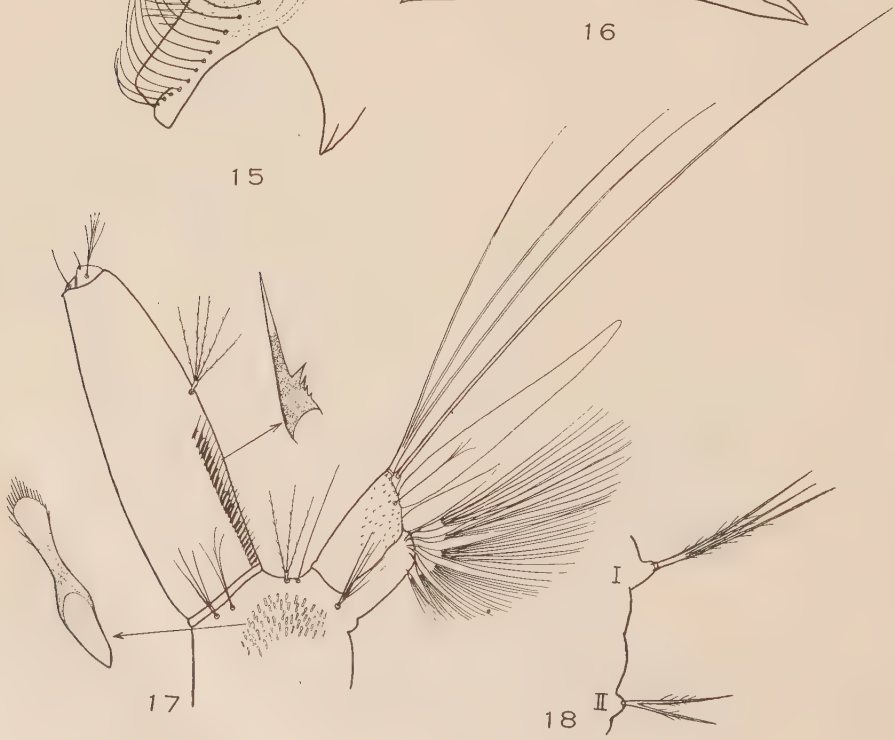

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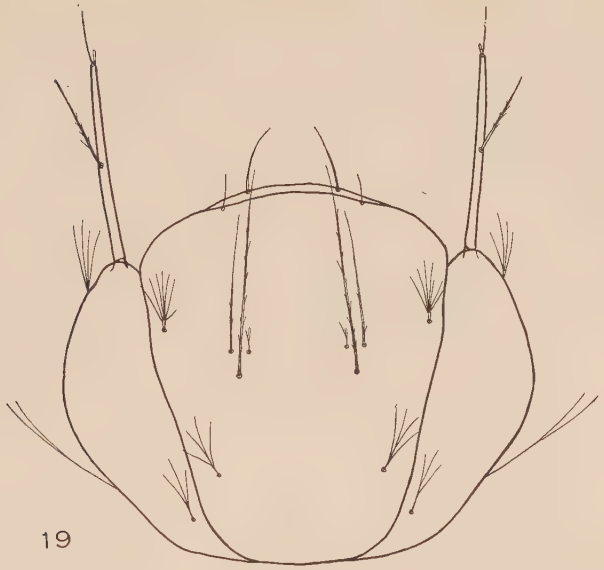

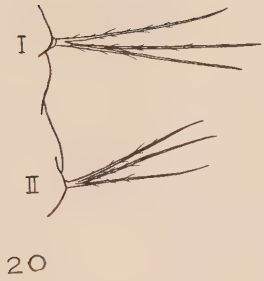

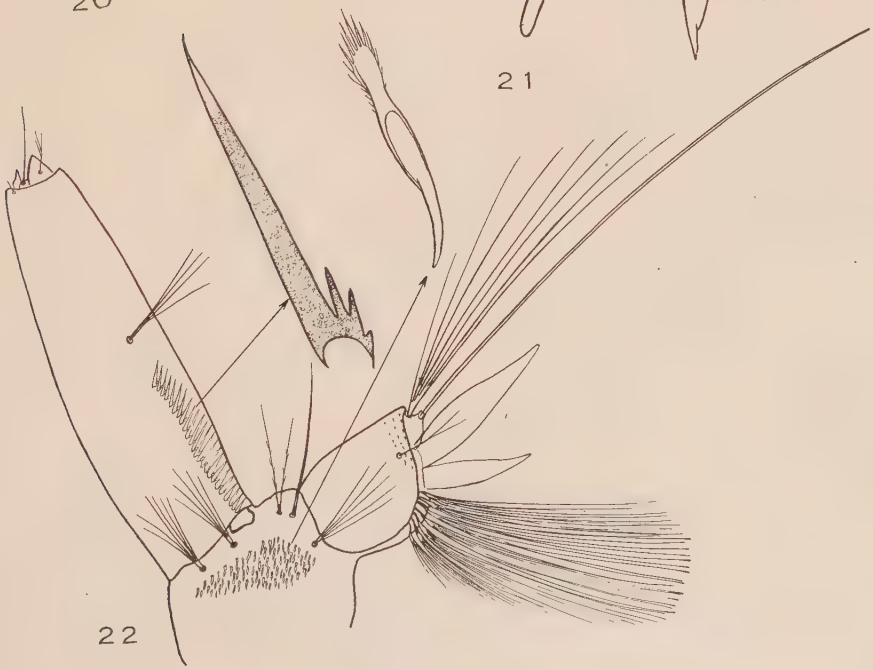

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